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(12) **United States Patent**
Gupta

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(54) **METHOD FOR TREATING PULMONARY DISORDERS WITH LIPOSOMAL AMIKACIN FORMULATIONS**

(75) Inventor: **Renu Gupta**, Moorestown, NJ (US)

(73) Assignee: **INSMED INCORPORATED**, Monmouth Junction, NJ (US)

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Primary Examiner — Gollamudi Kishore
(74) *Attorney, Agent, or Firm* — Cooley LLP

(57) **ABSTRACT**

Disclosed herein are methods of treating pulmonary disorders comprising administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein: the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

31 Claims, 21 Drawing Sheets

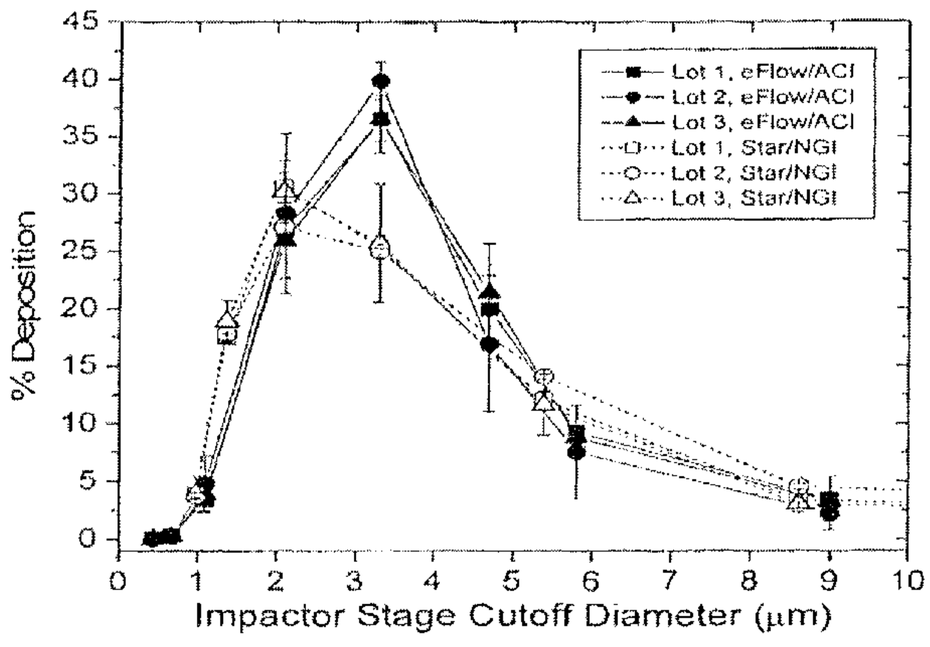

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Figure 1

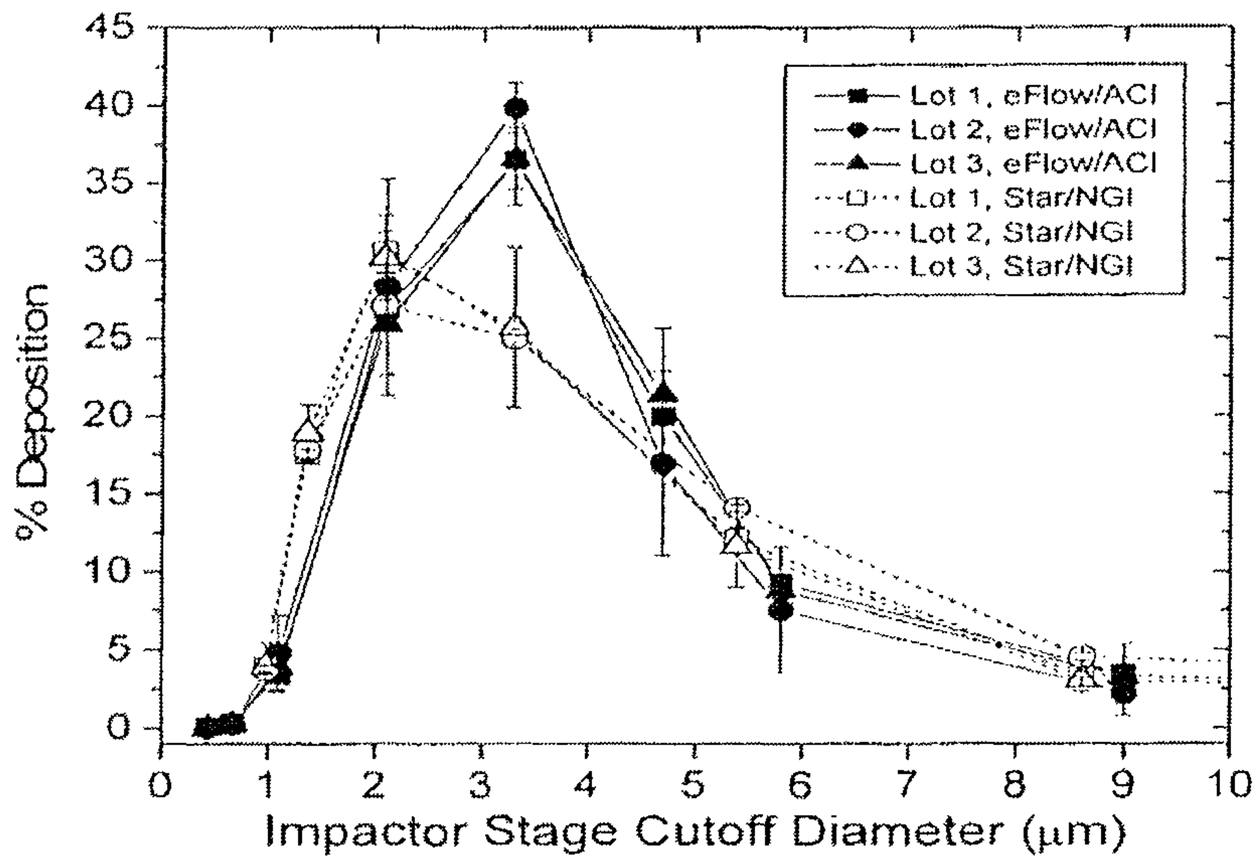

Lot 1, eFlow/ACI
Lot 2, eFlow/ACI
Lot 3, eFlow/ACI
Lot 1, Star/NGI
Lot 2, Star/NGI
Lot 3, Star/NGI
% Deposition
0
5
10
15
20
25
30
35
40
45
0
1
2
3
4
5
6
7
8
9
10
Impactor Stage Cutoff Diameter (μm)

Figure 2

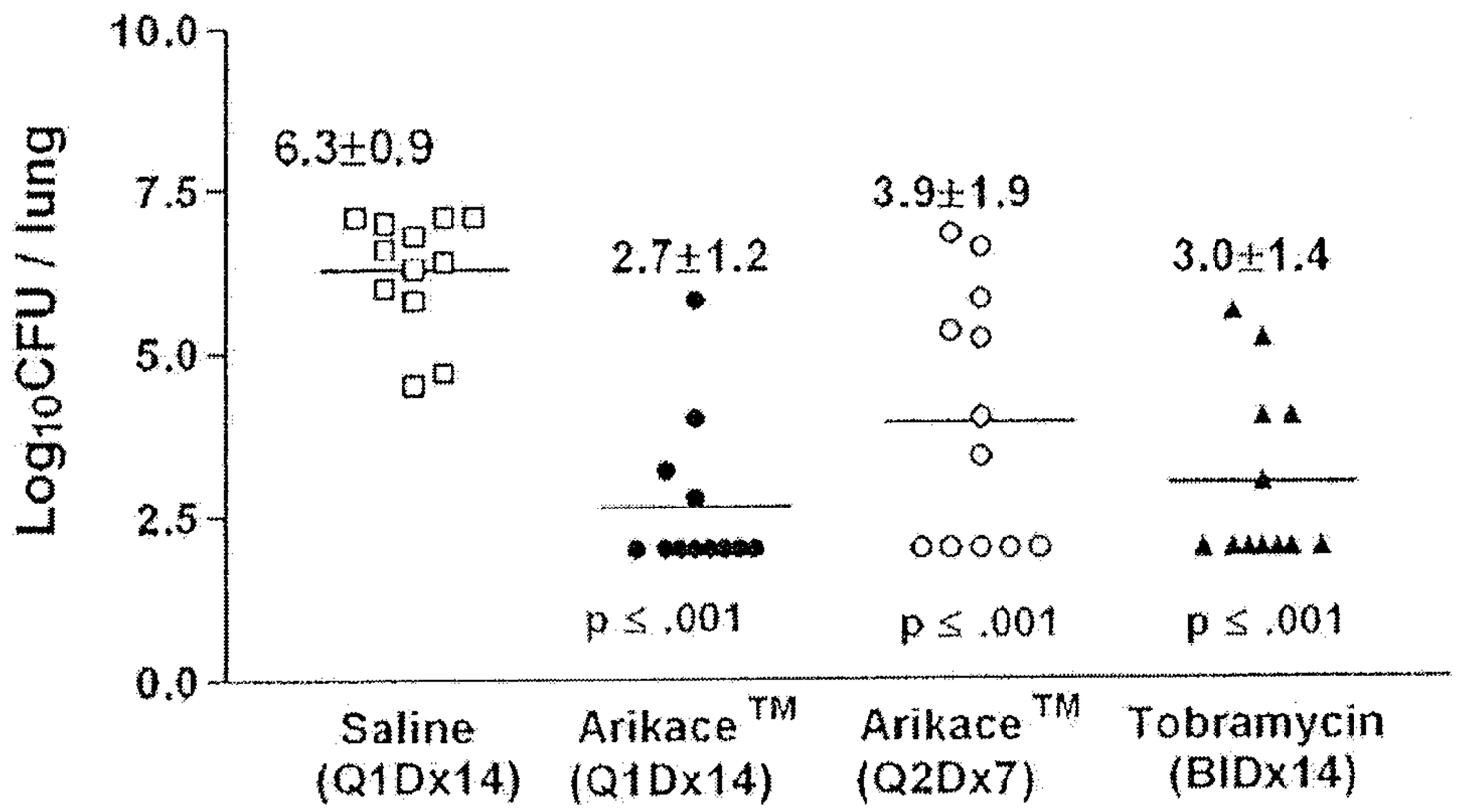

10.0
7.5
5.0
2.5
0.0
Log10CFU / lung
6.3±0.9
2.7±1.2
3.9±1.9
3.0±1.4
p ≤ .001
p ≤ .001
p ≤ .001
Saline (Q1Dx14)
Arikace™ (Q1Dx14)
Arikace™ (Q2Dx7)
Tobramycin (BIDx14)

Figure 3

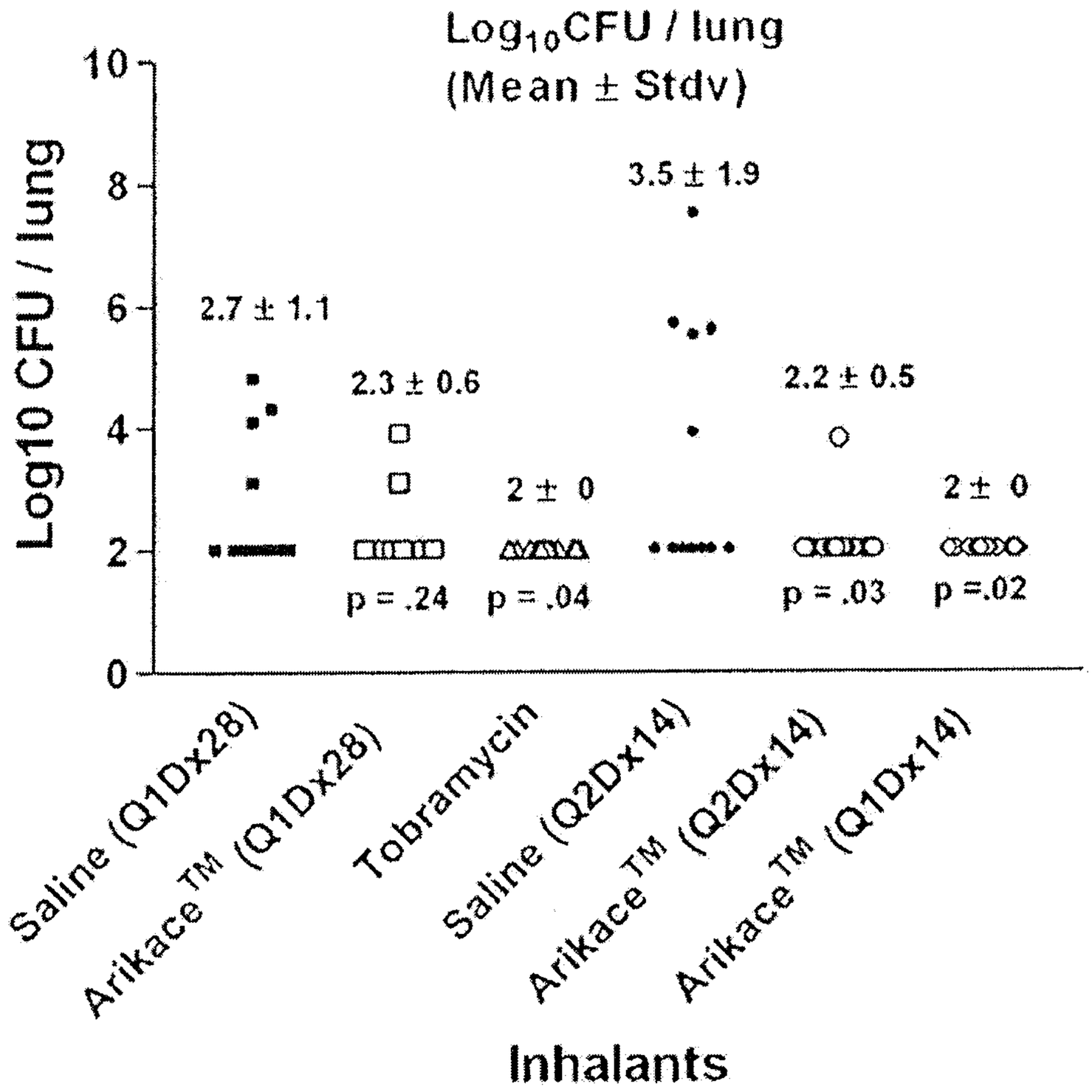

Log10CFU / lung
(Mean ± Stdv)
Log10 CFU / lung
10
8
6
4
2
0
2.7 ± 1.1
2.3 ± 0.6
2 ± 0
3.5 ± 1.9
2.2 ± 0.5
2 ± 0
p = .24
p = .04
p = .03
p =.02
Saline (Q1Dx28)
Arikace™ (Q1Dx28)
Tobramycin
Saline (Q2Dx14)
Arikace™ (Q2Dx14)
Arikace™ (Q1Dx14)
Inhalants

Figure 4

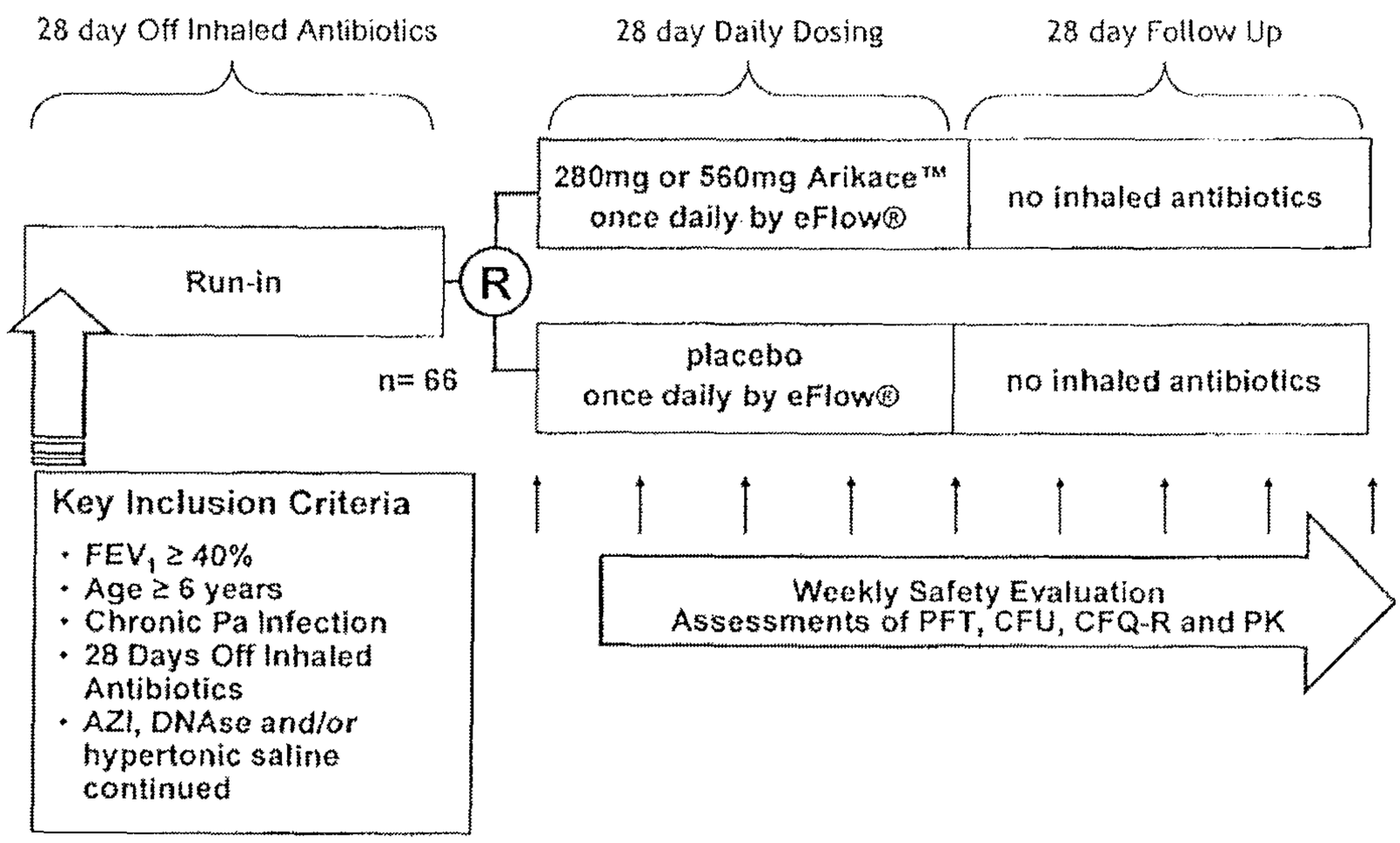
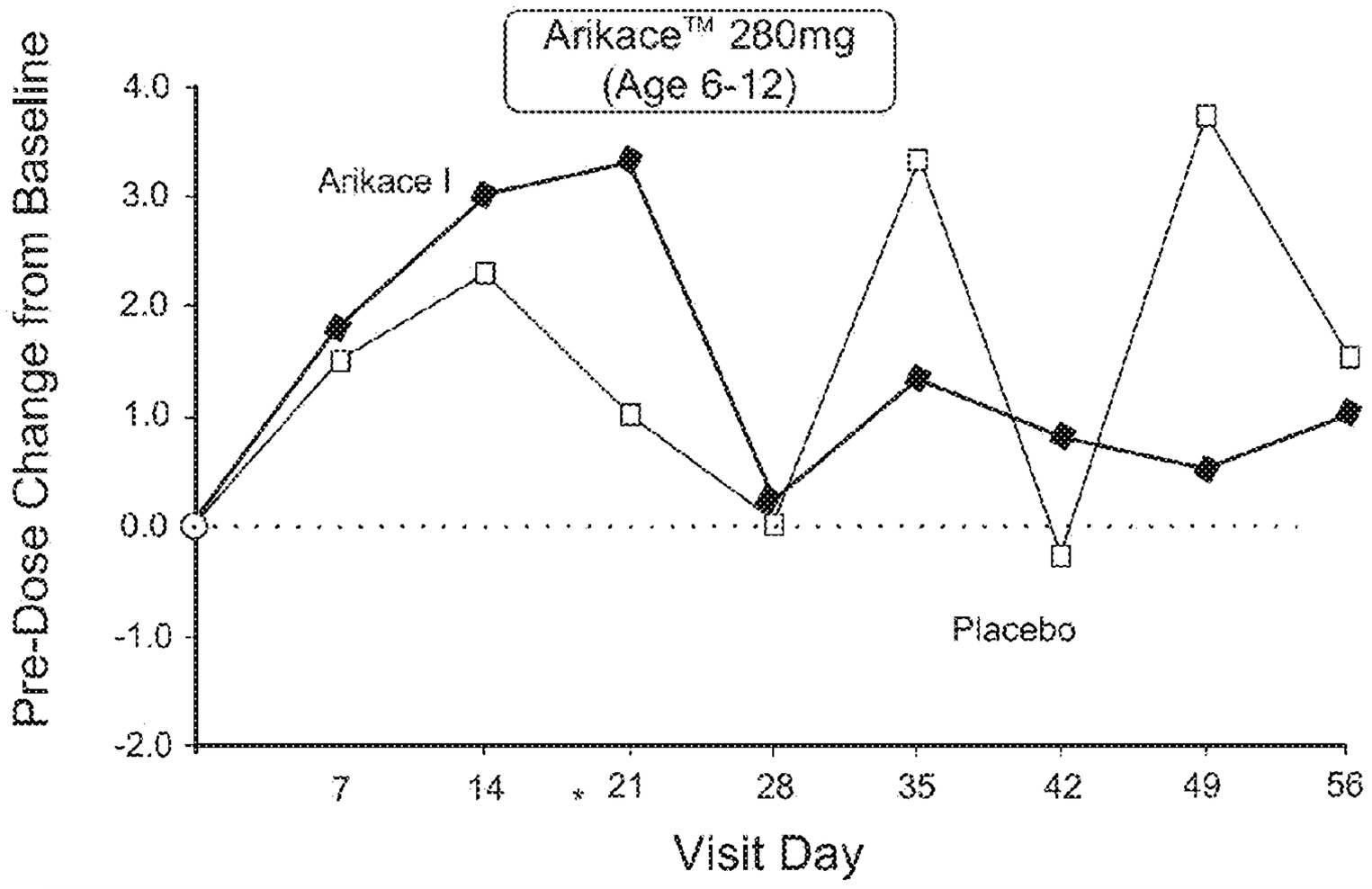

28 day Off Inhaled Antibiotics
28 day Daily Dosing
28 day Follow Up
280mg or 560mg Arikace™ once daily by eFlow®
no inhaled antibiotics
Run-in
R
n= 66
placebo once daily by eFlow®
no inhaled antibiotics
Key Inclusion Criteria
• FEV1 ≥ 40%
• Age ≥ 6 years
• Chronic Pa Infection
• 28 Days Off Inhaled Antibiotics
• AZI, DNAse and/or hypertonic saline continued
Weekly Safety Evaluation
Assessments of PFT, CFU, CFQ-R and PK

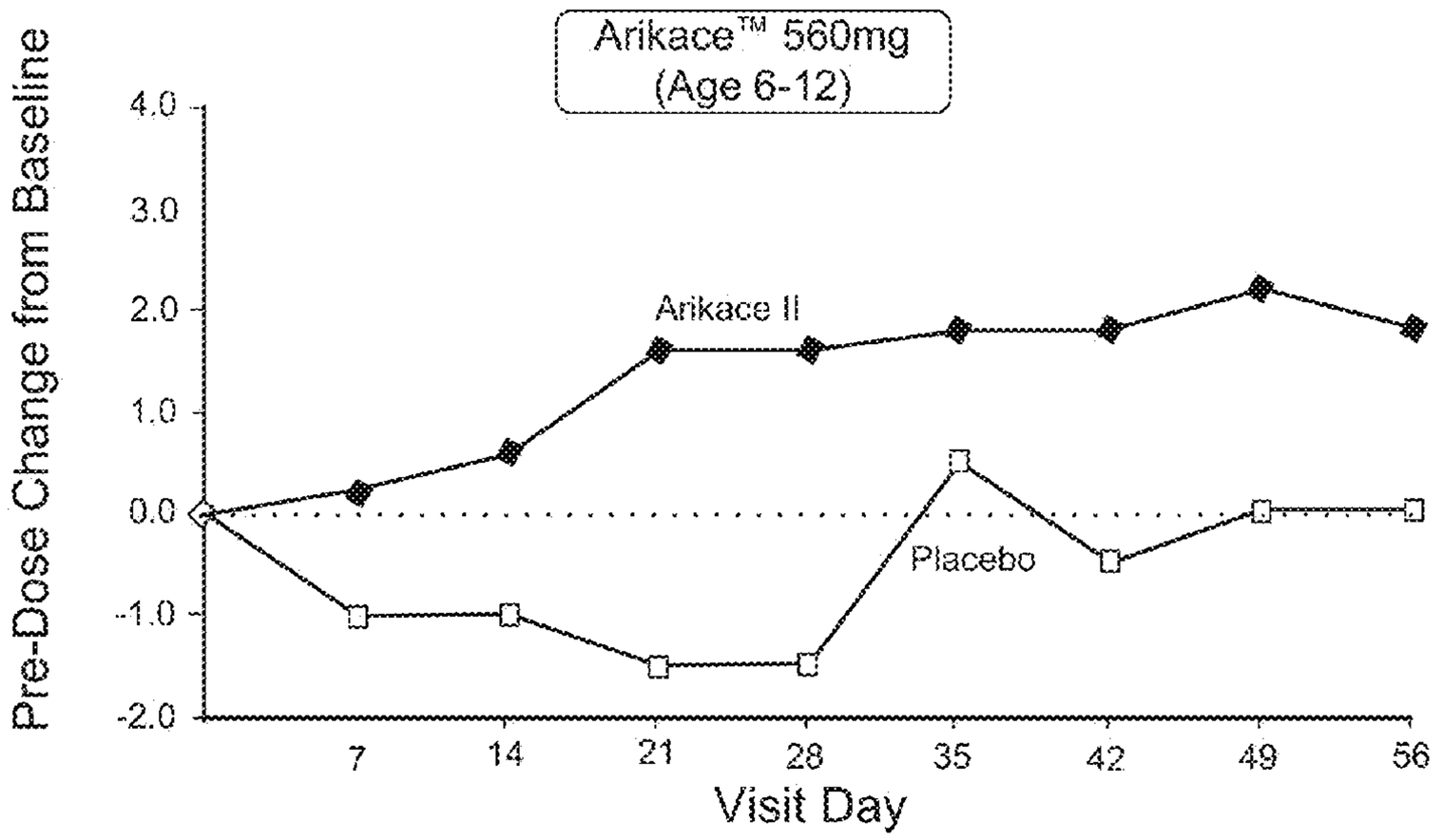
Figure 5



Arikace I	1.8	3.0 (1.4)	3.3	0.2 (4.1)	1.3	0.8 (0.5)	0.5	1.0
Placebo	1.5	2.3 (2.1)	1.0	0.0 (3.6)	3.3	-0.3 (3.3)	3.7	1.5

* Mean (SD)

Figure 6



Arikace II	0.2	0.6 (0.9)	1.6	1.6 (1.7)	1.8	1.8 (1.5)	2.2	1.8
Placebo	-1.0	-1.0 (0.0)	-1.5	-1.5 (0.7)	0.5	-0.5 (0.7)	0.0	0.0

* Mean (SD)

Figure 7a

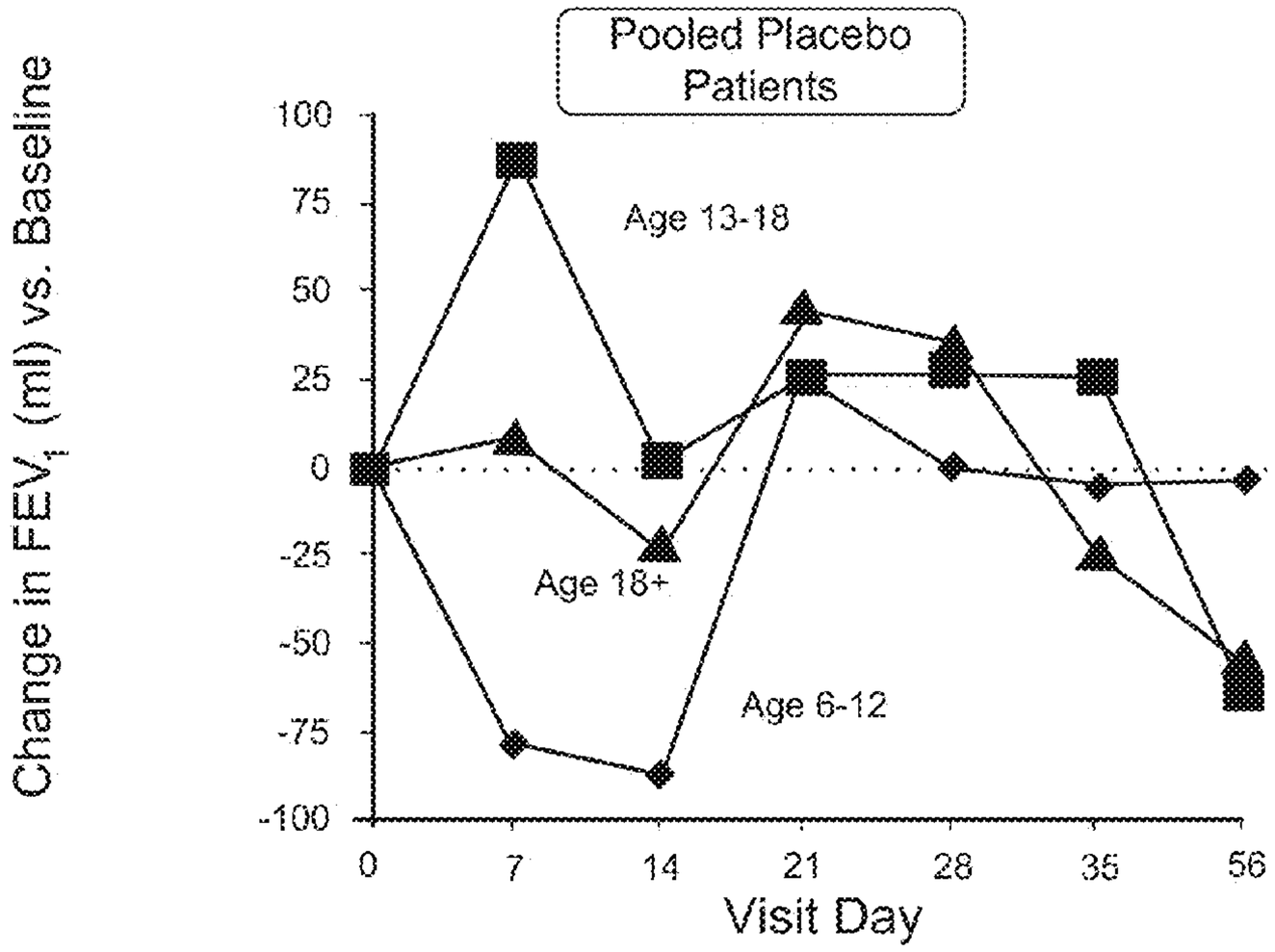


Figure 7b

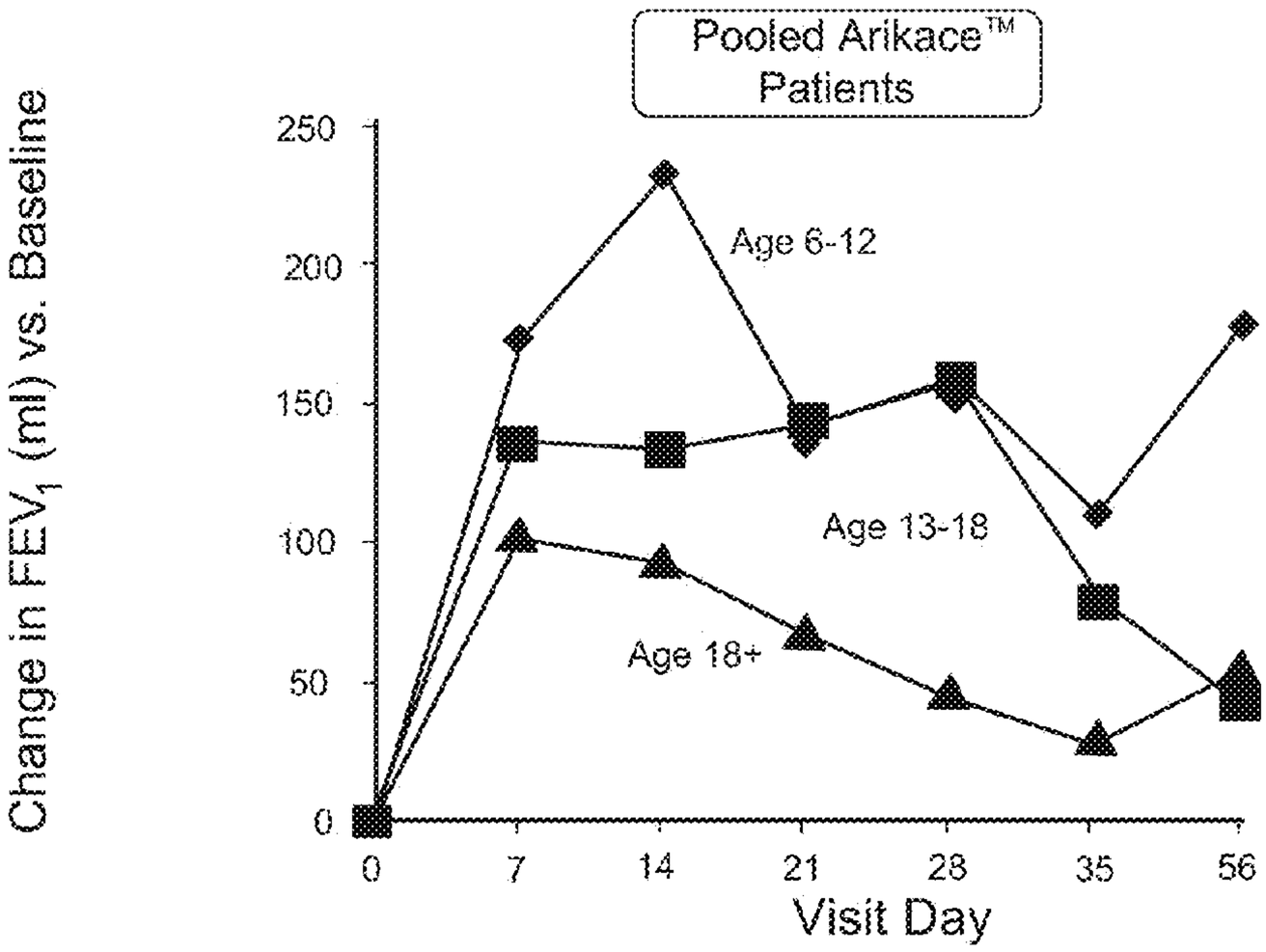
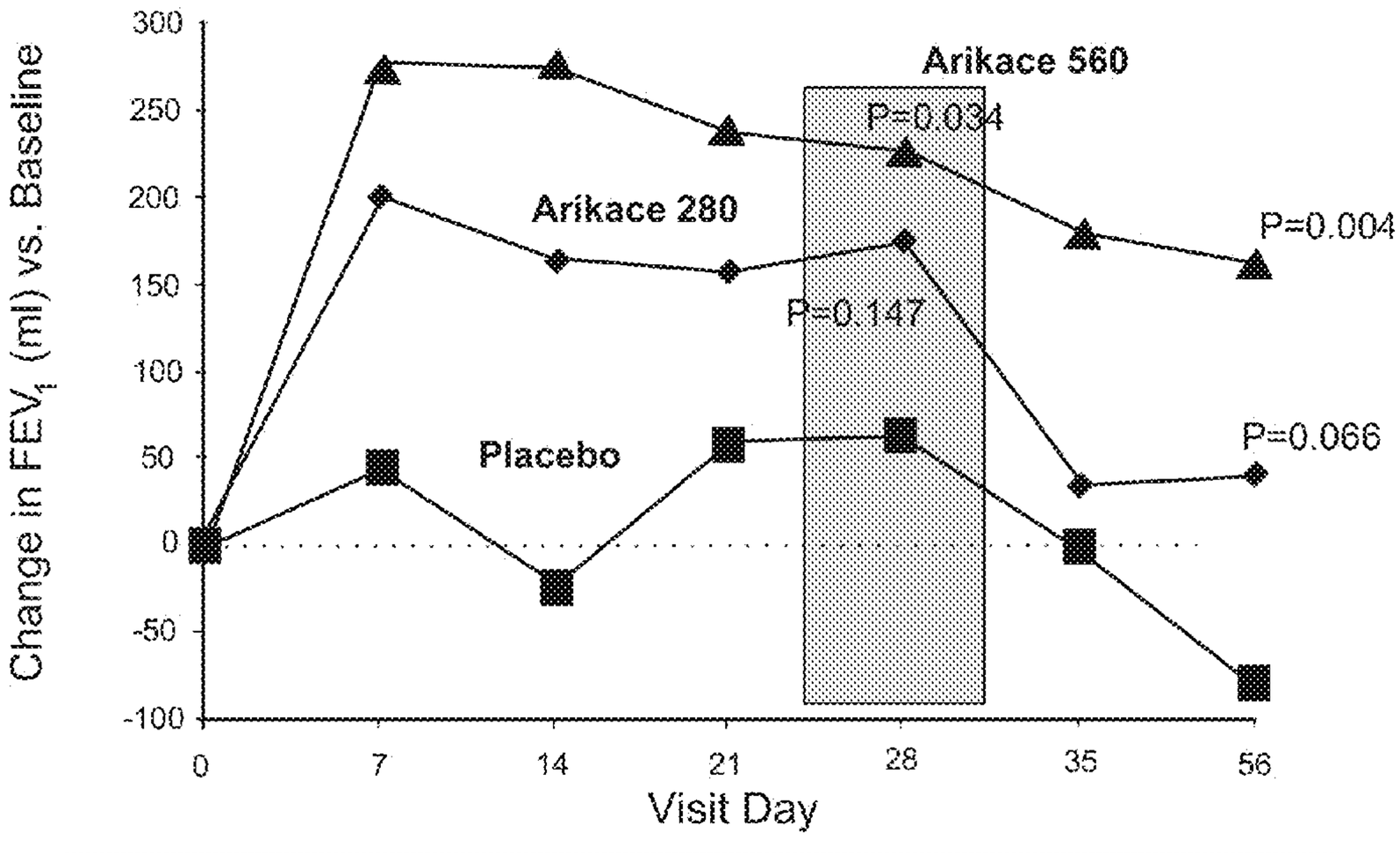


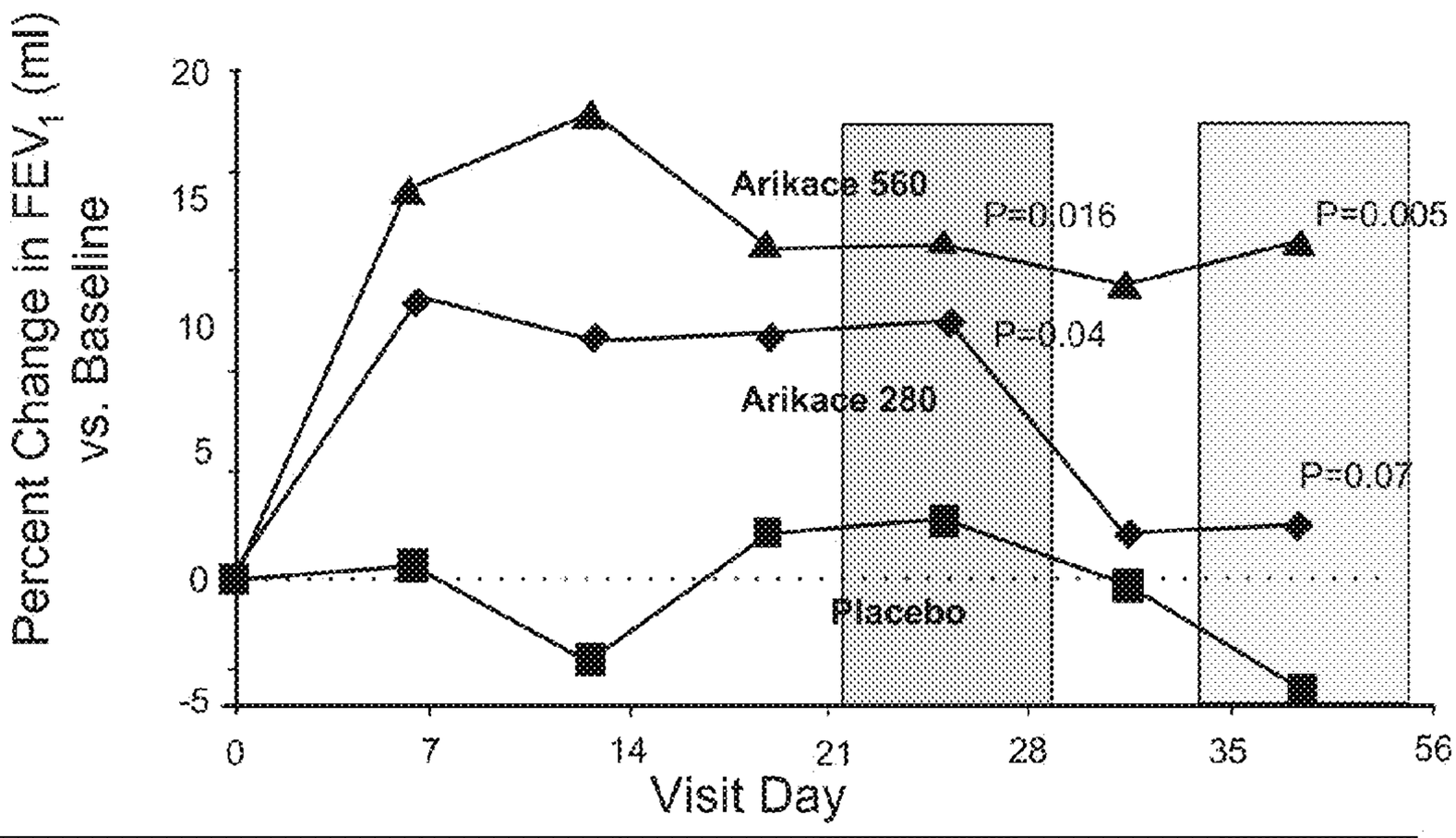
Figure 8



Arikace 560 *	276 (317)	275 (261)	237 (243)	226 (229)	179 (245)	162 (292)
Arikace 280 *	200 (220)	164 (240)	157 (222)	174 (235)	33 (176)	39 (176)
Placebo *	44 (201)	-24 (218)	58 (224)	62 (253)	-3 (238)	-82 (229)

* Mean (SD)

Figure 9



Arikace 560 *	15.4% (16.5)	18.4% (21.3)	13.2% (15.3)	13.2% (15.2)	11.5% (16.4)	13.2% (24.3)
Arikace 280 *	10.9% (10.6)	9.4% (12.6)	9.6% (12.5)	10.1% (12.8)	1.7% (9.0)	2.0% (8.6)
Placebo *	0.6% (11.7)	-3.2% (12.2)	1.8% (10.9)	2.2% (11.8)	-0.3% (12.0)	-4.4% (13.0)

* Mean (SD)

Figure 10

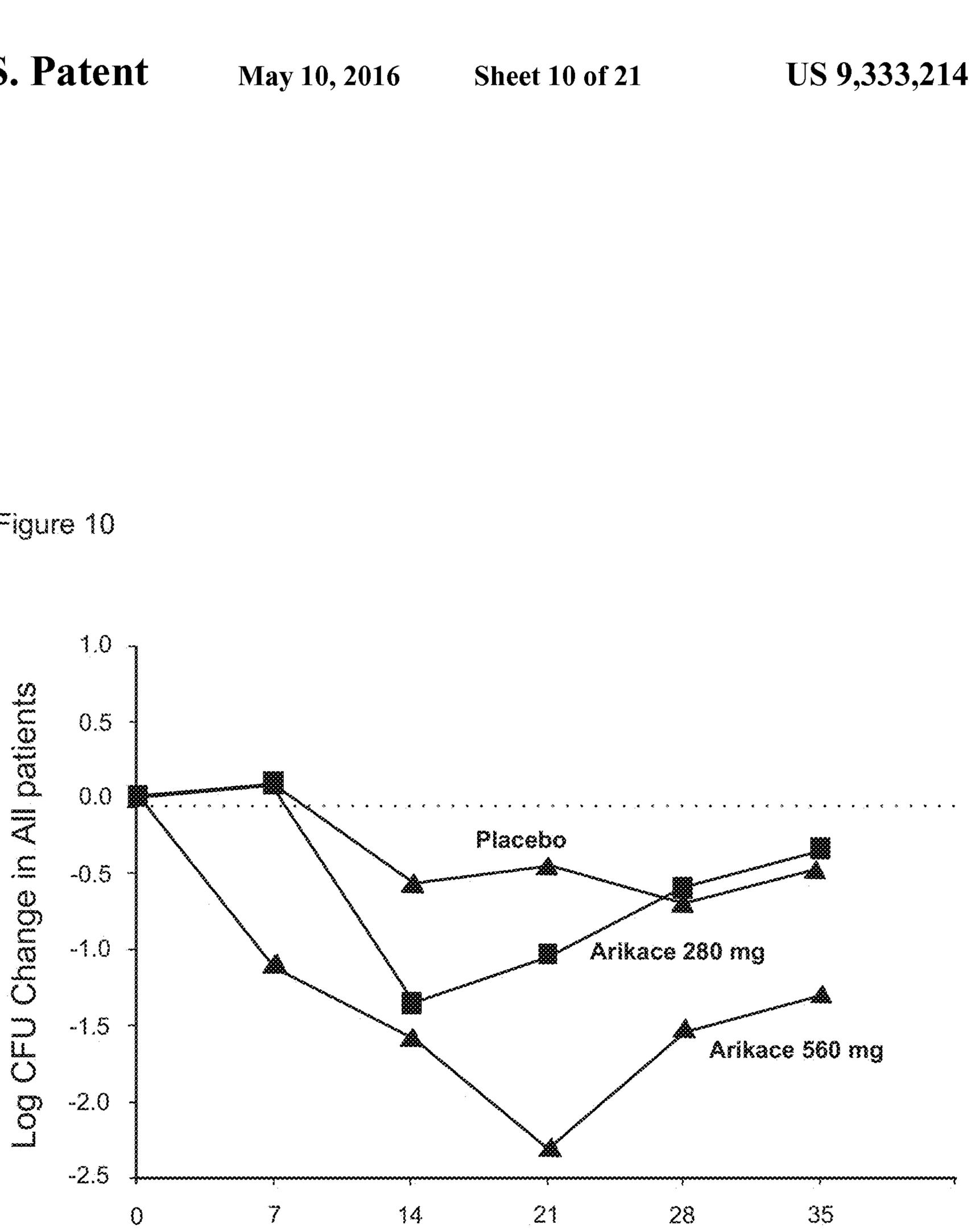
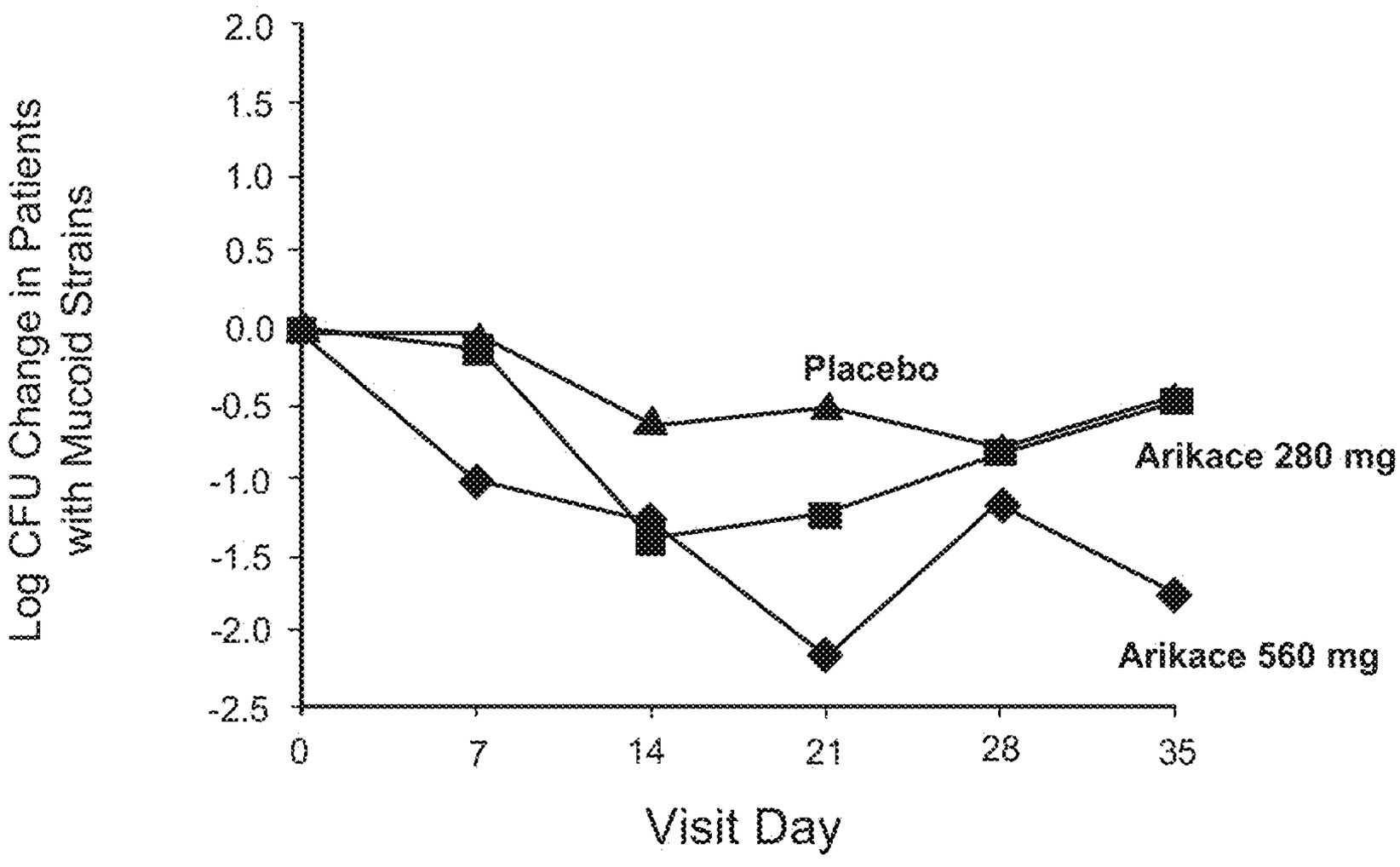

Log CFU Change in All patients
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Placebo
Arikace 280 mg
Arikace 560 mg
0
7
14
21
28
35
Visit Day

Figure 11



280 mg Arikace	-0.141	-1.391	-1.263	-0.816	-0.480
560 mg Arikace	-0.993	-1.263	-2.138	-1.163	-1.788
Placebo	-0.033	-0.600	-0.510	-0.772	-0.449

Figure 12

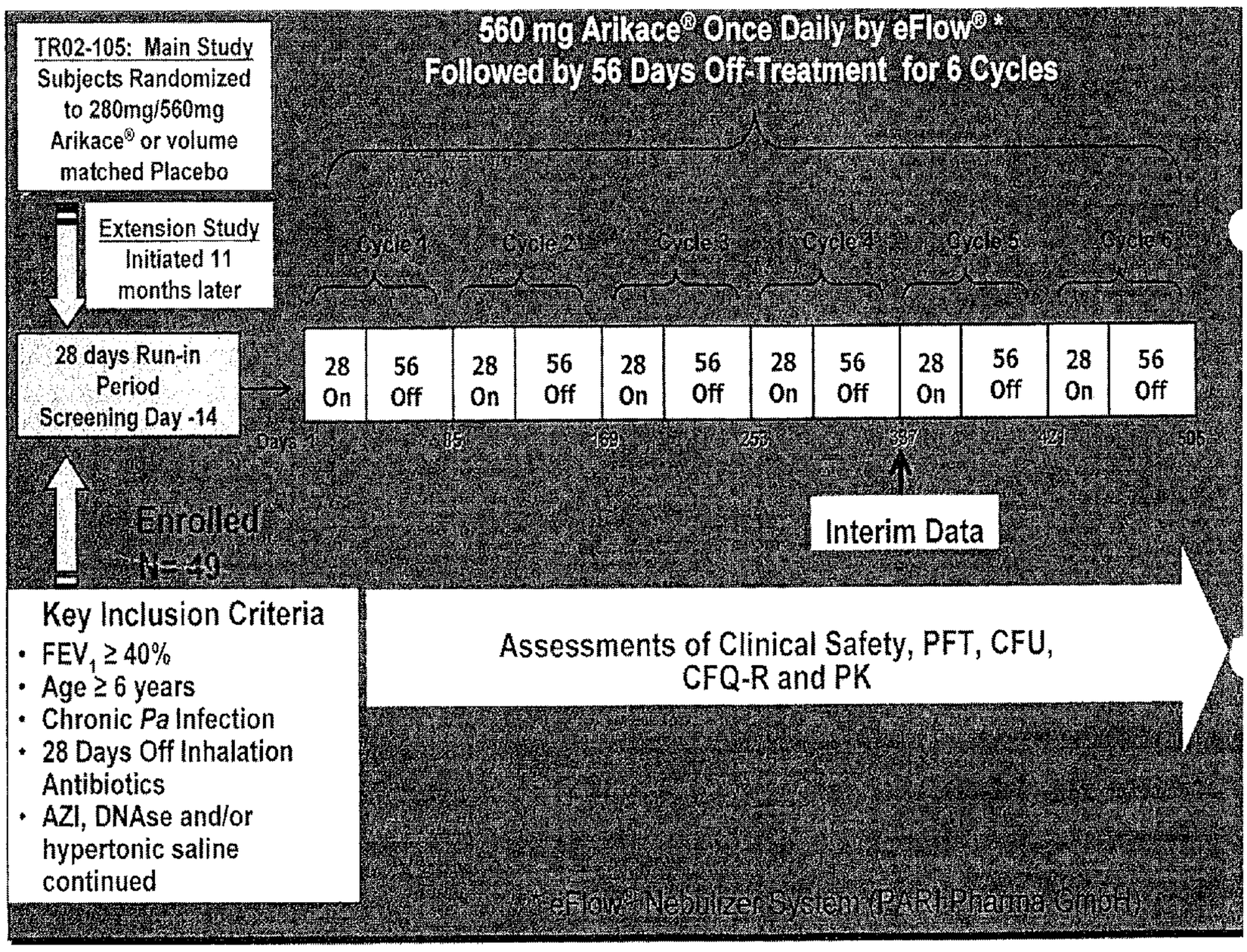

560 mg Arikace® Once Daily by eFlow® *
Followed by 56 Days Off-Treatment for 6 Cycles
TR02-105: Main Study
Subjects Randomized to 280mg/560mg Arikace® or volume matched Placebo
Extension Study
Initiated 11 months later
28 days Run-in Period
Screening Day -14
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6
28 On
56 Off
Days 1
85
169
253
337
421
505
Interim Data
Enrolled
N=49
Key Inclusion Criteria
• FEV1 ≥ 40%
• Age ≥ 6 years
• Chronic Pa Infection
• 28 Days Off Inhalation Antibiotics
• AZI, DNAse and/or hypertonic saline continued
Assessments of Clinical Safety, PFT, CFU, CFQ-R and PK
* eFlow® Nebulizer System (PARI Pharma GmbH)

Figure 13

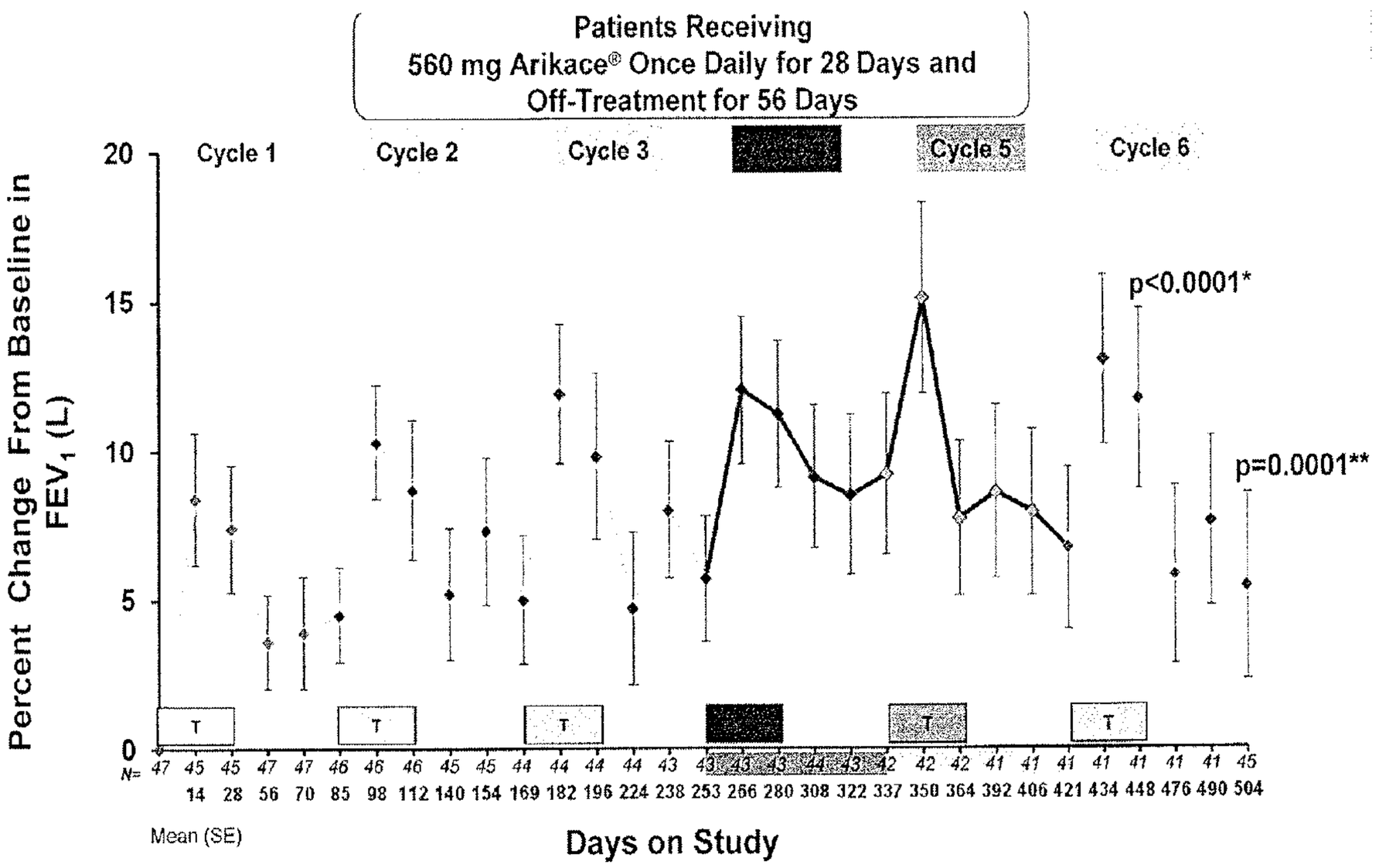
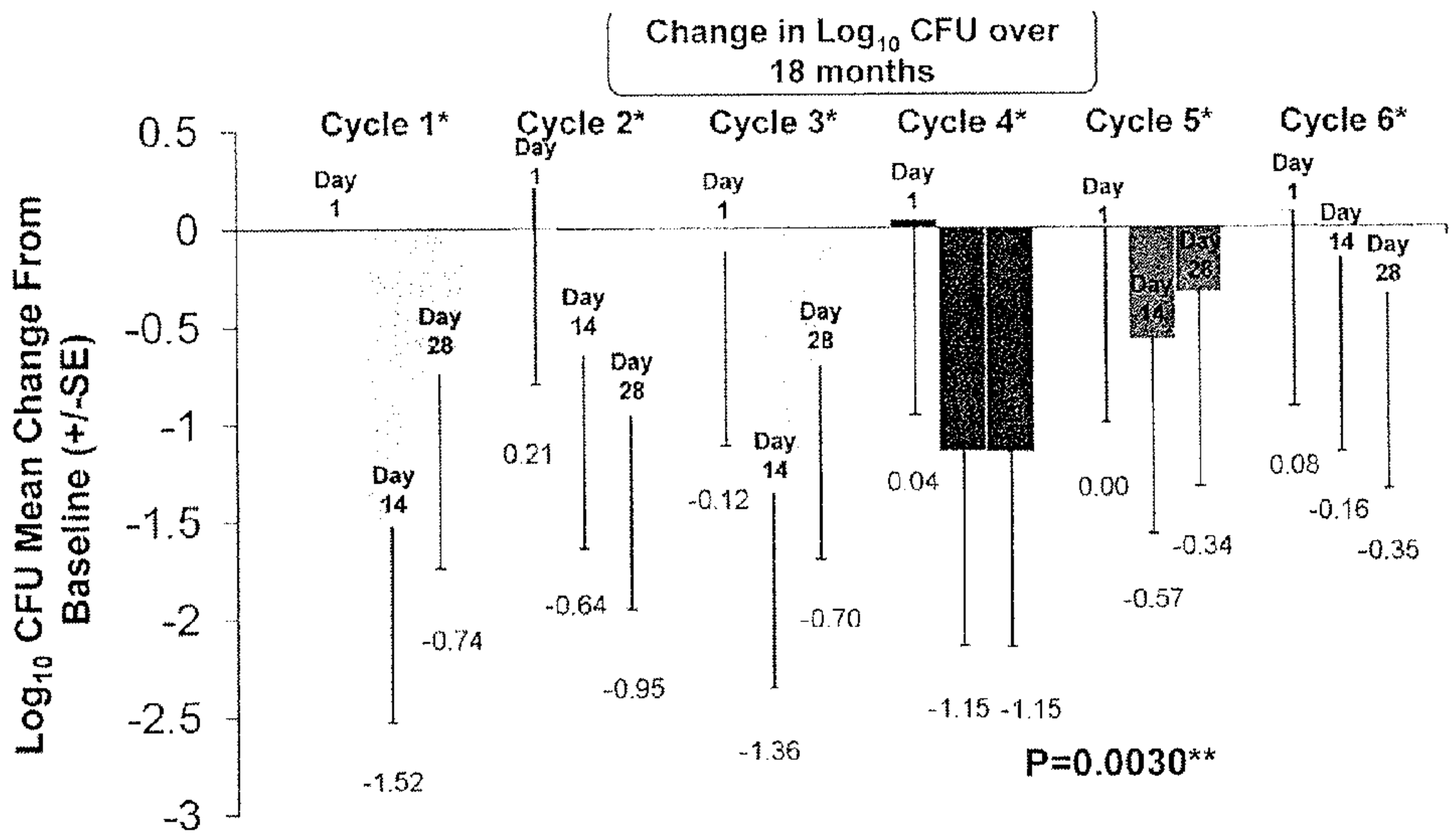

Patients Receiving
560 mg Arikace® Once Daily for 28 Days and
Off-Treatment for 56 Days
Cycle 1
Cycle 2
Cycle 3
Cycle 5
Cycle 6
p<0.0001*
p=0.0001**
Percent Change From Baseline in FEV1 (L)
20
15
10
5
0
T
N= 47 45 45 47 47 46 46 46 45 45 44 44 44 44 43 43 43 43 44 43 42 42 42 41 41 41 41 41 41 41 45
14 28 56 70 85 98 112 140 154 169 182 196 224 238 253 266 280 308 322 337 350 364 392 406 421 434 448 476 490 504
Mean (SE)
Days on Study

Figure 14



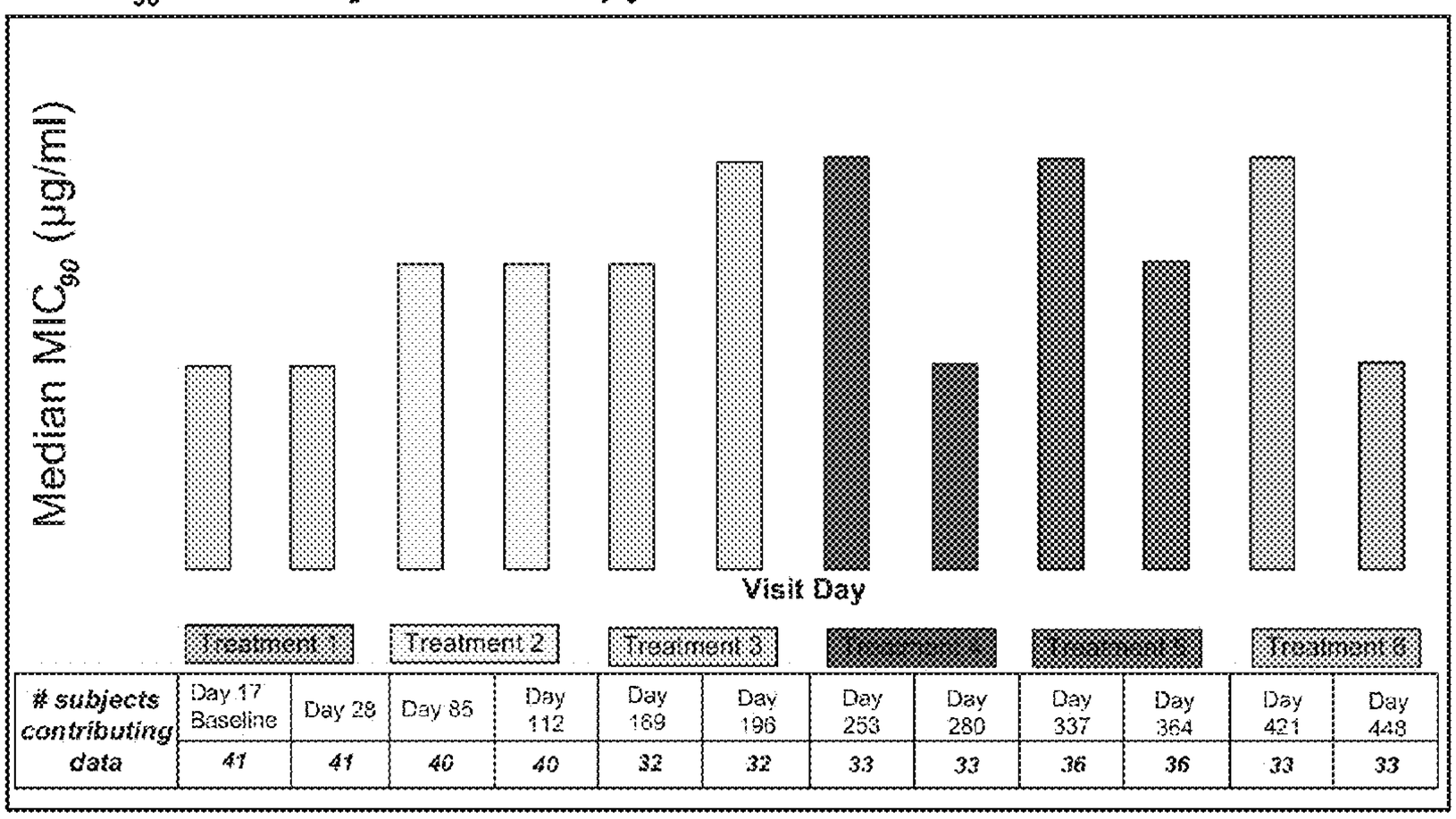
* Each cycle consists of 28 days of once daily treatment followed by 56 days off-treatment

* Day 1 values for Cycles 2, 3, 4, 5 and 6 are change from Baseline (Day 1 value of Cycle 1)

** Reduction in Log_{10} CFU is statistically significant during Cycles 1-6

Figure 15

An open label extension study of Arikace demonstrated no significant change in MIC_{90} over six cycles of therapy



# subjects contributing data	Day 17 Baseline	Day 28	Day 85	Day 112	Day 169	Day 196	Day 253	Day 280	Day 337	Day 364	Day 421	Day 448
	41	41	40	40	32	32	33	33	36	36	33	33

Figure 16

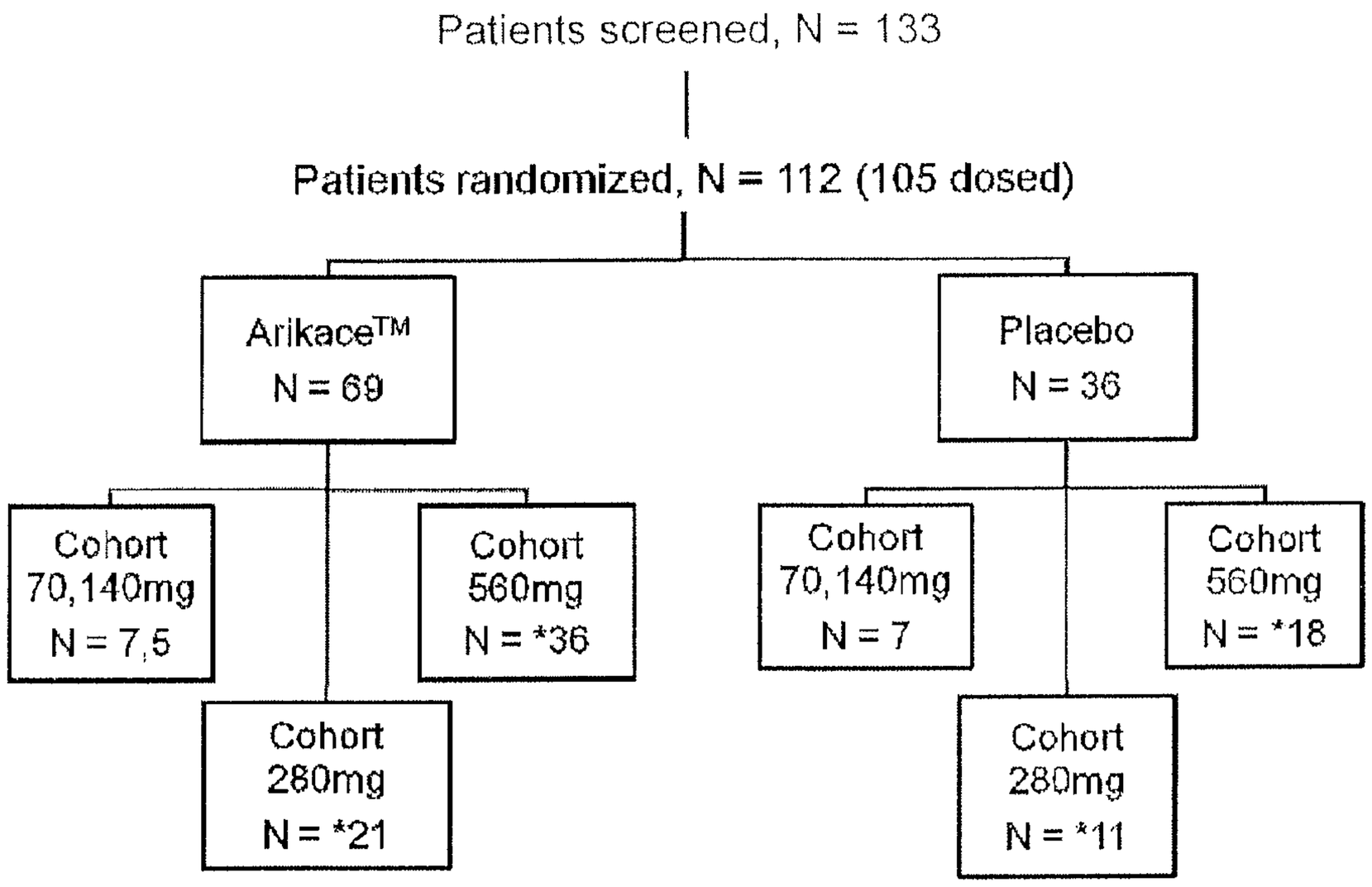

Patients screened, N = 133
Patients randomized, N = 112 (105 dosed)
Arikace™ N = 69
Placebo N = 36
Cohort 70,140mg N = 7,5
Cohort 560mg N = *36
Cohort 280mg N = *21
Cohort 70,140mg N = 7
Cohort 560mg N = *18
Cohort 280mg N = *11

Figure 17

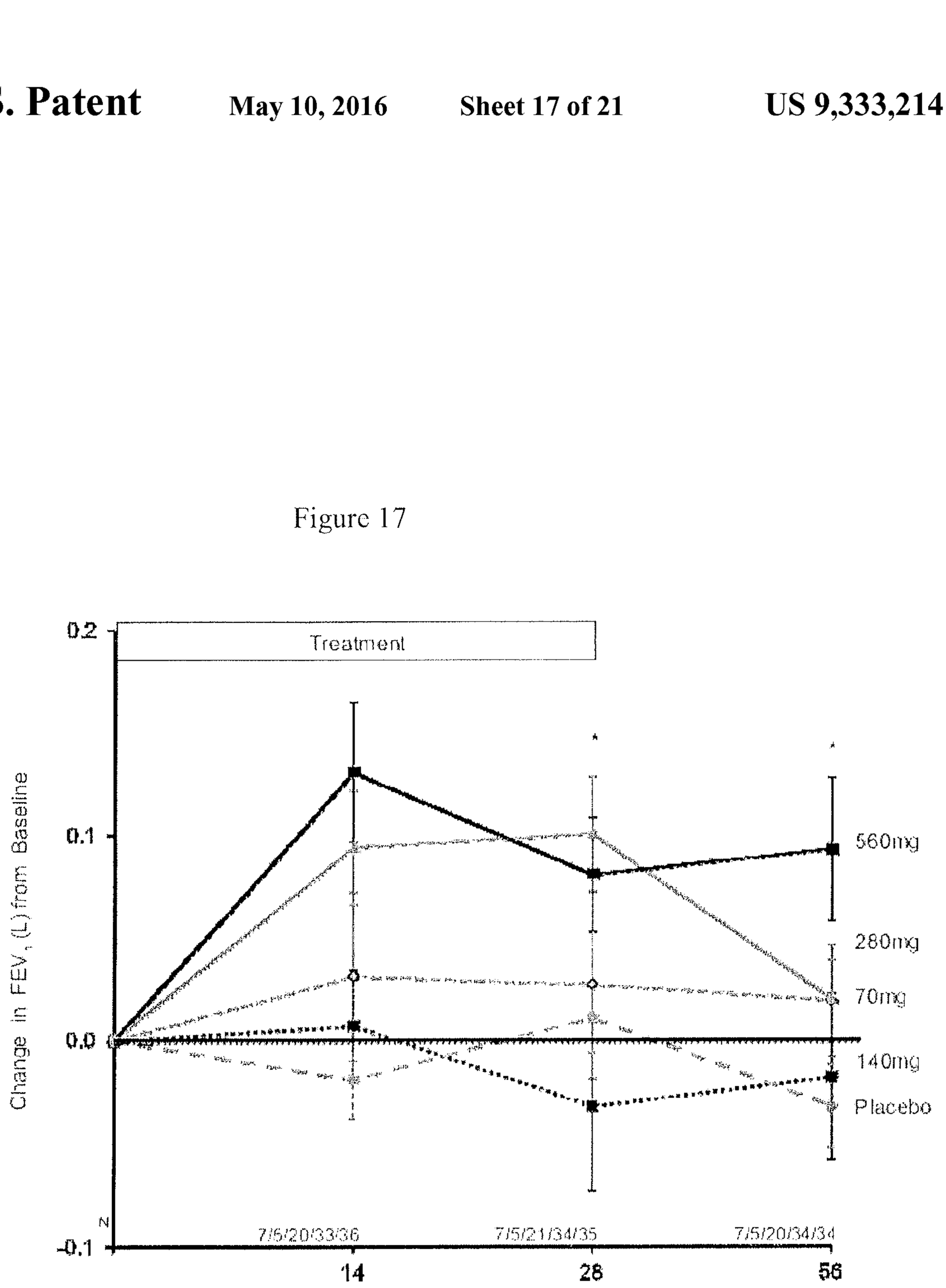

Treatment
0.2
0.1
0.0
-0.1
Change in FEV1 (L) from Baseline
560mg
280mg
70mg
140mg
Placebo
N
7/5/20/33/36
7/5/21/34/35
7/5/20/34/34
14
28
56
Study Day

Figure 18

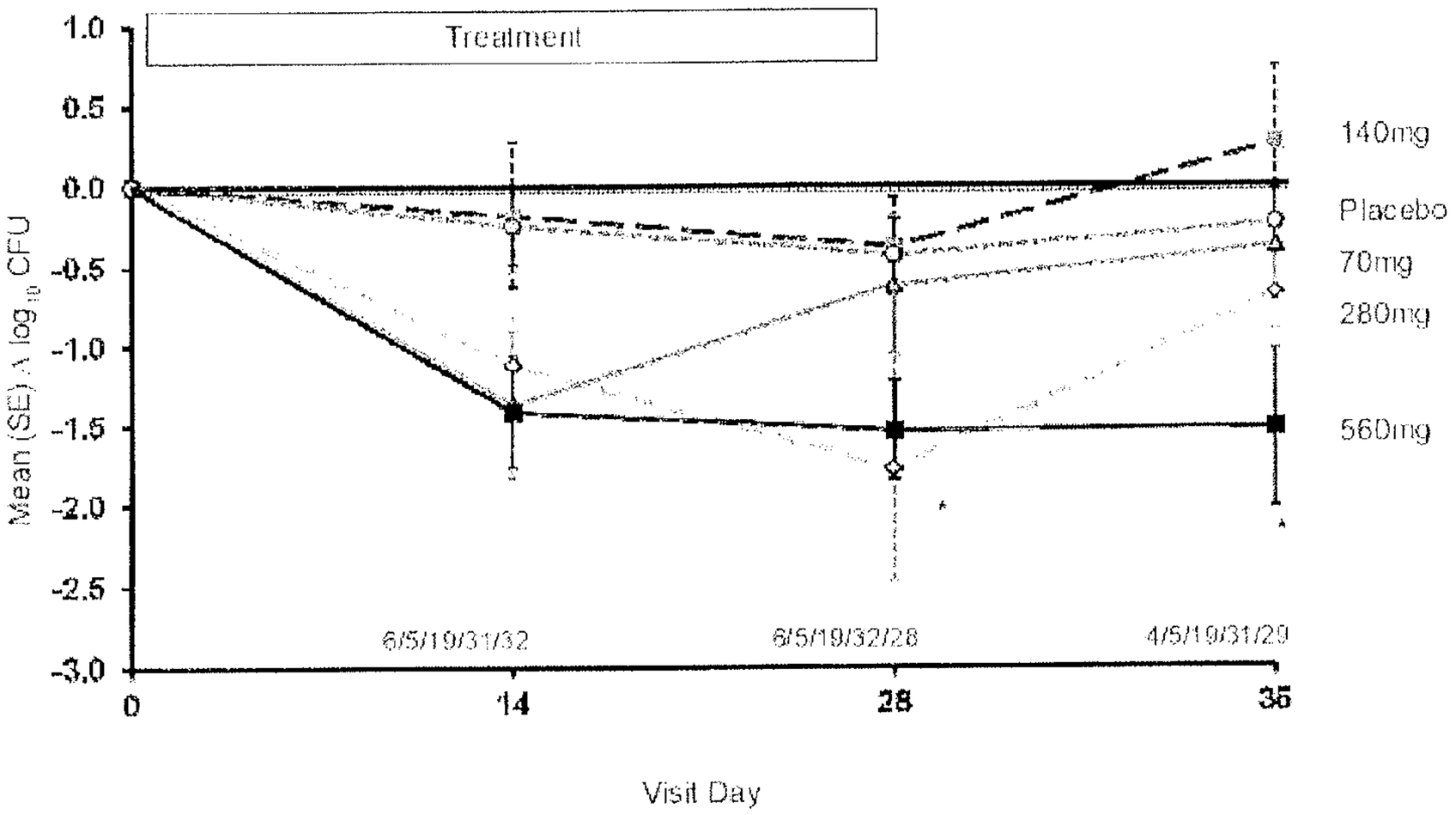
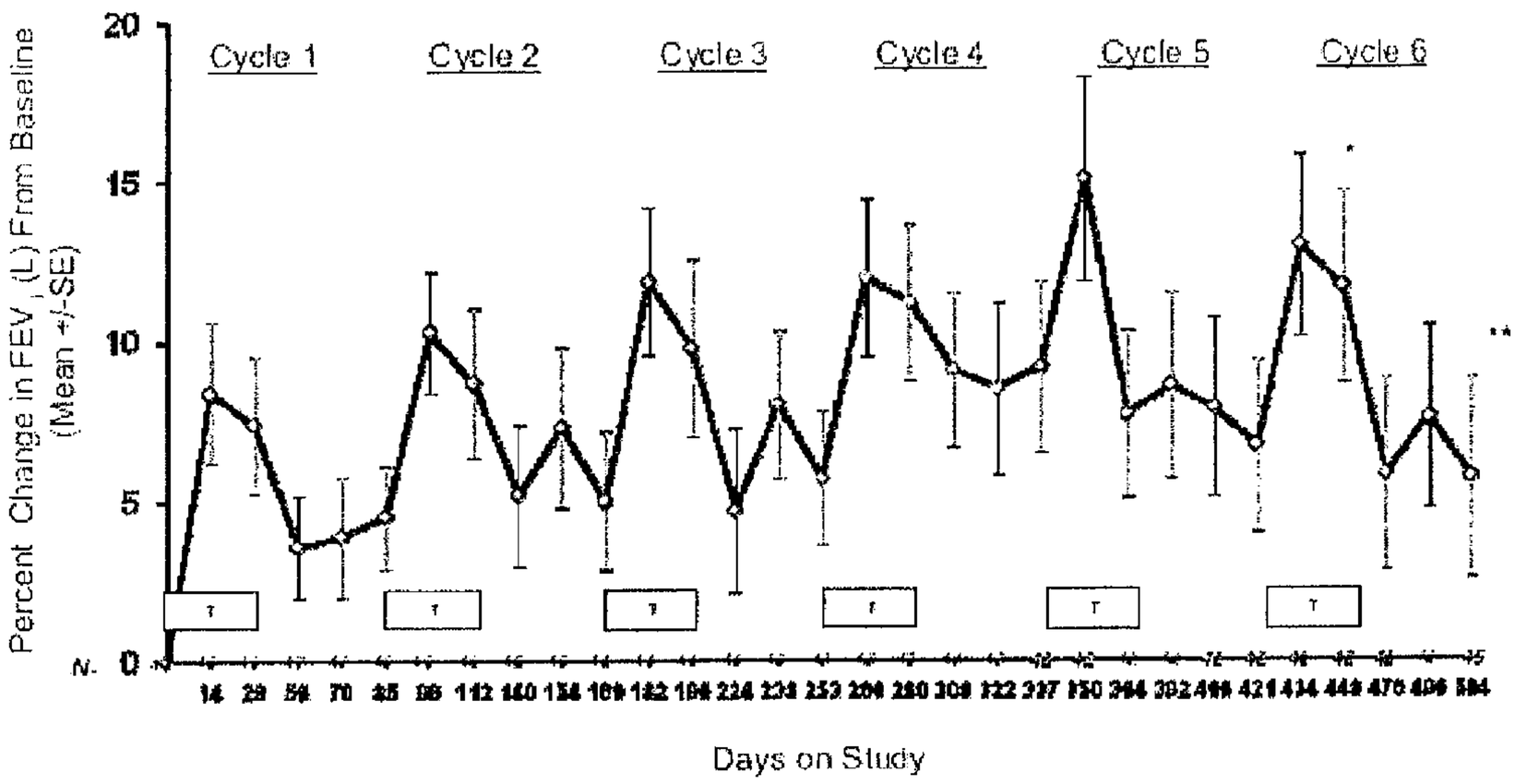

Treatment
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Mean (SE) Δ log10 CFU
6/5/19/31/32
6/5/19/32/28
4/5/19/31/29
0
14
28
35
Visit Day
140mg
Placebo
70mg
280mg
560mg

Figure 19



T = Treatment Period

Figure 20

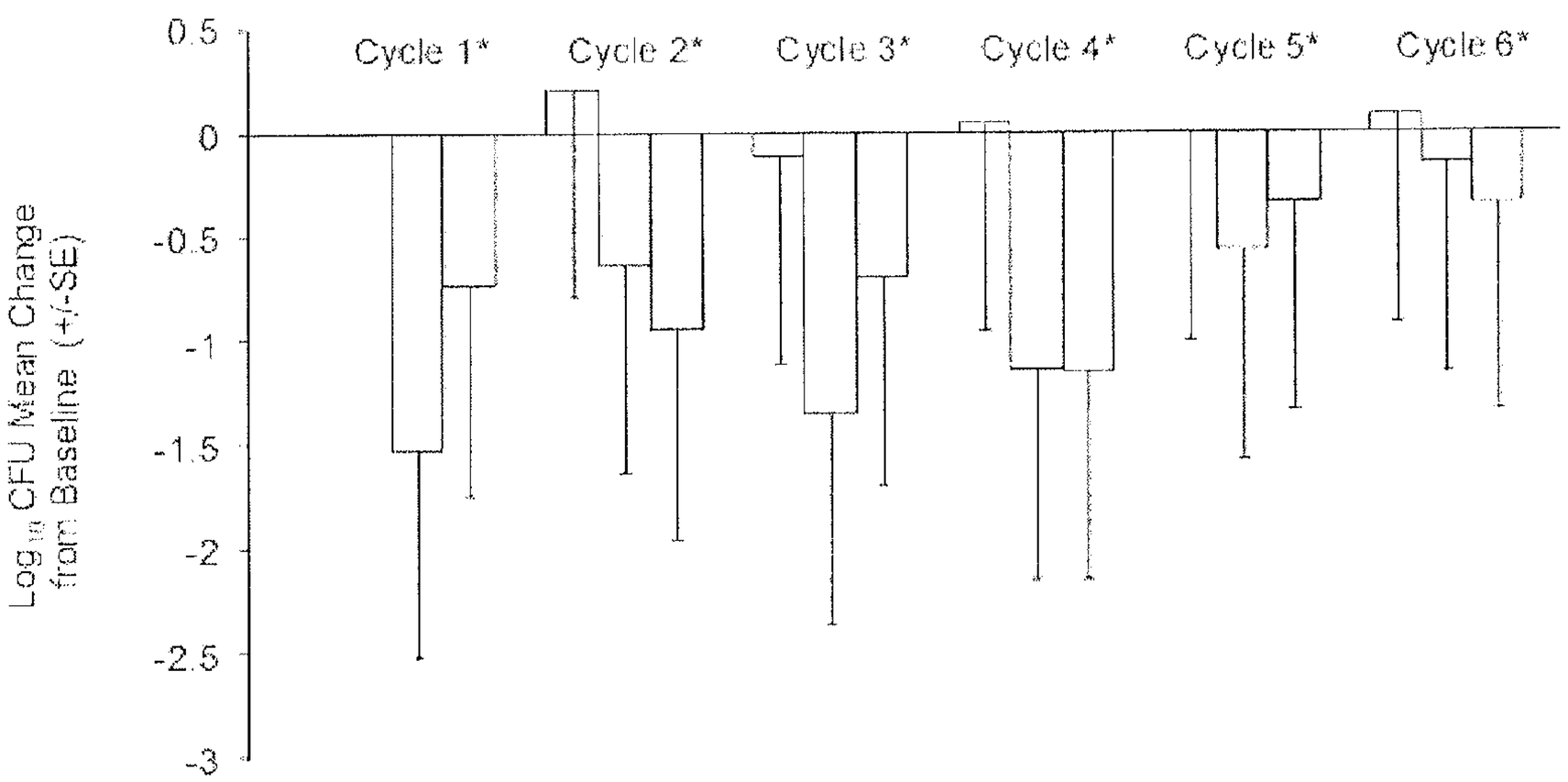
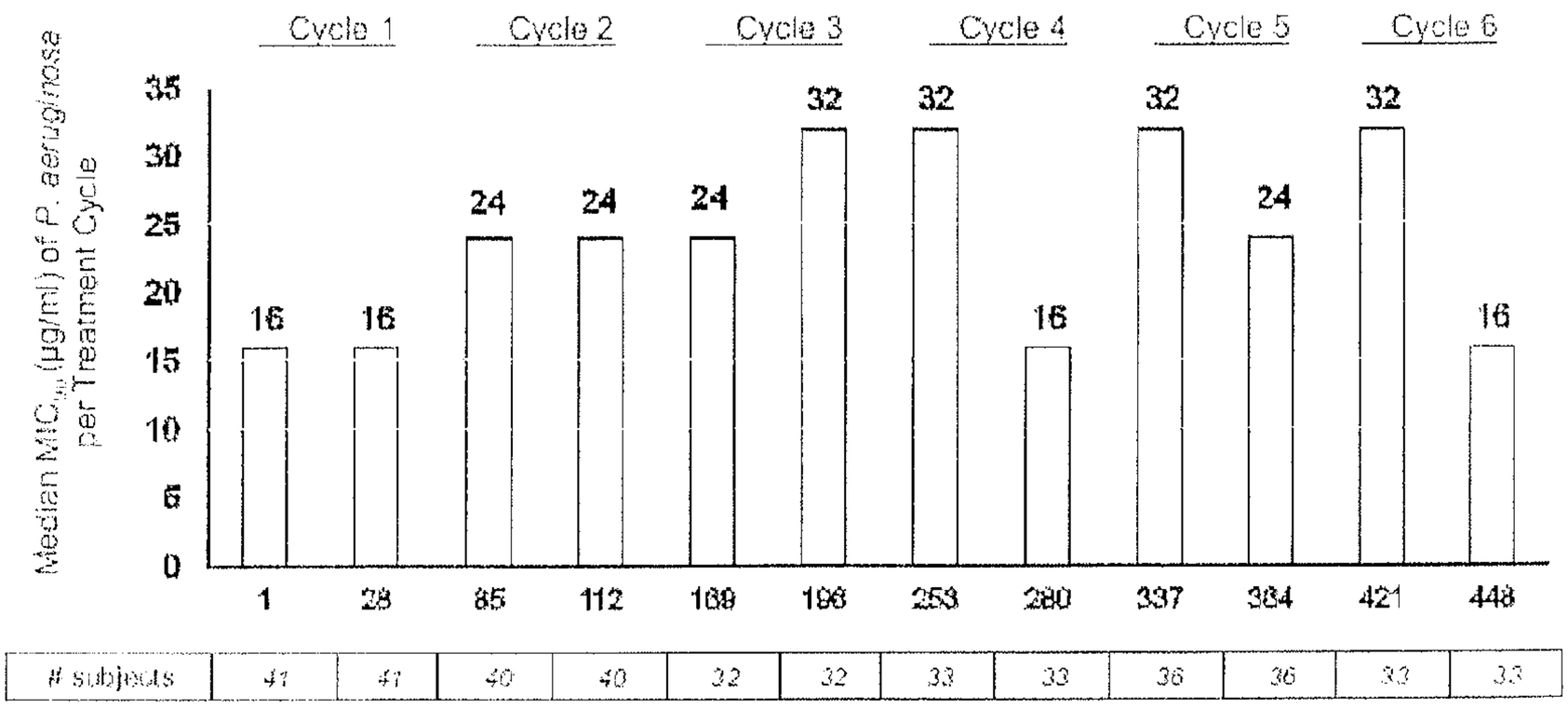

Cycle 1*
Cycle 2*
Cycle 3*
Cycle 4*
Cycle 5*
Cycle 6*
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
Log10 CFU Mean Change from Baseline (+/-SE)

Figure 21


Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6
Median MIC90 (µg/ml) of P. aeruginosa per Treatment Cycle
35
30
25
20
15
10
5
0
16
16
24
24
24
32
32
16
32
24
32
16
1
28
85
112
169
196
253
280
337
364
421
448
subjects
41
41
40
40
32
32
33
33
36
36
33
33
Visit Day

METHOD FOR TREATING PULMONARY DISORDERS WITH LIPOSOMAL AMIKACIN FORMULATIONS

RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application Ser. No. 61/514,574, filed Aug. 3, 2011, and is a continuation in part of U.S. application Ser. No. 13/480,246, filed May 24, 2012, which claims priority from U.S. Provisional Application Ser. No. 61/489,940, filed May 25, 2011. U.S. application Ser. No. 13/480,246 is a continuation in part of U.S. application Ser. No. 12/250,412, filed on Oct. 13, 2008, which is a continuation in part of International Application Serial No. PCT/US08/062868, filed on May 7, 2008, which claims priority from U.S. Provisional Application Ser. No. 60/916,342, filed on May 7, 2007. Each of the forgoing applications is incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

Cystic fibrosis (CF), also called mucoviscidosis, is an autosomal, recessive, hereditary disease of the exocrine glands. It affects the lungs, sweat glands and the digestive system, causing chronic respiratory and digestive problems. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. It is the most common fatal autosomal recessive diseases amongst Caucasians.

The first manifestation of CF is sometimes meconium ileus, occurring in 16% of infants who develop CF. Other symptoms of CF manifest during early childhood. Both lungs and pancreas produce abnormally viscous mucus. This mucus begins to build up and starts to clog the opening to the pancreas and the lungs. Pulmonary problems start from the constant presence of thick, sticky mucus and are one of the most serious complications of CF. The mucus in the lungs can become a growth medium for bacteria, resulting in chronic respiratory infections and eventual permanent damage to the lung tissue. During the end stage of CF, the patient experiences increased chest congestion, activity intolerance, increased crackles, and increased cough, which often contains sputum mixed with blood (hemoptysis) due to the bronchiole bleeding from the lung arteries. A chronic and loose sounding cough is common in people with CF. These thick secretions also obstruct the pancreas, preventing digestive enzymes from reaching the intestines to help break down and absorb food. Frequent and foul smelling stools are often an early sign of CF along with fatty oil that is visible in the stool. This can compromise growth and overall nutrition if proper treatment to aid digestion is not utilized early in life. As lung function deteriorates, CF patients can develop pulmonary hypertension, chronic bronchitis, and chronic dilation of the bronchioles (bronchiectasis). Lung abscess are very common. Death usually occurs from severe infection, pneumonia, or heart failure.

Cystic fibrosis is exclusively heritable as both parents must carry the recessive genes for a child to acquire the disease. At the genetic level, cystic fibrosis is most often the result of an in-frame deletion of three base pairs in the DNA. Cystic fibrosis results from the production of an abnormal form of a protein called cystic fibrosis transmembrane conductance regulator (CFTR). CFTR functions in transporting chloride ions across epithelial cells found in the lung and intestinal tract. In CF patients, CFTR does not function properly, causing accumulation of ions inside epithelial cells. Since water follows ions by osmosis, this results in water depletion and viscous mucus on the surface of alveoli. The most common

CFTR protein abnormality is a mutation termed ΔF508, which is characterized by the 3-bp deletion of the DNA base-pair sequence at chromosome location 7q31.1-31.2 that codes for the amino acid, phenylalanine.

In addition to pulmonary infections, most people with CF also have problems with digestion, particularly the digestion of fats. This leads to malabsorption and difficulty gaining and maintaining weight, which in turn affects overall health. This is due to the abnormally sticky mucus that blocks the release of digestive enzymes from the pancreas. Pancreatic insufficiency is treated with supplemental enzymes. Usually water-miscible forms of the fat-soluble vitamins A, D, E, and K are required as the decreased fat absorption can lead to deficiencies of these vitamins.

CF patients also have an increased incidence of diabetes mellitus because of the pancreatic blockage. The chronic blocking causes the Islets of Langerhans to degrade over time and decrease insulin production, causing hyperglycemia. There is also evidence that patients with CF become more resistant to the insulin that is produced, this can be triggered by infections or treatment with corticosteroids. Diabetes in CF patients is commonly referred to as CFRD, cystic fibrosis related diabetes. A typical diabetic diet is not feasible and therefore insulin doses are instead adjusted to fit the typical high-calorie/high-fat CF diet.

Many CF patients, to some degree, experience the widening of the tips of their fingers, known as "clubbing". The condition affects fingers and toes, and results in the tip of the digit being round and enlarged. This can also be seen in people with COPD or severe heart disease. Since people with CF are prone to poor absorption of nutrients, osteoporosis can occur in early adulthood due to low bone density. It is important for people with CF to have regular dual energy X-ray absorptiometry (DEXA) scans to measure bone density and begin treatment if needed. When diagnosed early, treatment can help prevent more serious complications.

Some CF patients have hearing loss as a side effect of long-term use of the -mycin/-micin group of drugs, such as Tobramycin, which is used to combat lung infections. Although this side-effect is well-known and understood, these particular antibiotics are of high value in the treatment of CF patients, and often the hearing loss must be considered a necessary trade-off in order to preserve life and health. CF occurs primarily in individuals of central and western European origin. In the United States, the median age at death has increased from 8.4 years of age in 1969 to 14.3 years of age in 1998. The mean age of death has increased from 14 years in 1969 to 32.4 years of age in 2003 (Cystic Fibrosis Foundation). A major contributor to the significant increase in life expectancy is improved antibiotic treatment of chronic respiratory tract infections in CF subjects (Goss and Rosenfeld 2004) as well as improved nutrition and earlier diagnosis.

A major factor in the respiratory health of CF subjects is acquisition of chronic *Pseudomonas aeruginosa* infections. The infection rate with *P. aeruginosa* increases with age and by age 18 years, 80% of CF subjects in the U.S. are infected. The difficulties treating this infection are multifactorial, including poor penetration of antibiotics into sites of infection including mucus plugs, inactivation of antibiotics by CF sputum, growth of bacteria in a biofilm, changes in phenotype including conversion to a mucoid form of *P. aeruginosa*, and emergence of multi-drug resistance (Chmiel and Davis 2003; Gibson, Burns et al. 2003). The cornerstone of pulmonary therapy is optimizing treatment of *P. aeruginosa* as infection with this pathogen is associated with a poor clinical outcome (Doring, Conway et al. 2000; Chmiel and Davis 2003; Gibson, Burns et al. 2003; Gibson, Emerson et al. 2003).

One of the current approaches to management of chronic *P. aeruginosa* infection in humans with CF includes the use of suppressive therapy with inhaled tobramycin (TOBI®). Inhaled tobramycin, 300 mg, administered twice a day for cycles of 28 days followed by 28 days off drug has been shown to reduce *P. aeruginosa* colony counts, increase FEV_1% predicted, reduce hospitalizations, and decrease antibiotic use (Ramsey, Pepe et al. 1999). Nevertheless, patients have to be dosed twice a day for approximately 15-20 minute inhalation periods per dose.

Daily chest physiotherapy and aerosol breathing treatments are very commonly prescribed for CF patients. Typical physical therapy involves manual chest percussion (pounding), positive pressure techniques and/devices or possibly using a device such as the ThAIRapy Vest or the Intrapulmonary Percussive Ventilator (IPV) to achieve the same effect: loosening of the thick mucus. Aerosolized medicines commonly given include albuterol, ipratropium bromide and Pulmozyme to loosen secretions and decrease inflammation. It was found that CFers who surf were healthier; consequently, some hospitals use a nebulized 6%-10% Saline solution on those CFers who do not have asthma to loosen the secretions. Inhaled aminoglycoside antibiotics are sometimes given to fight infections. A number of pharmacological agents that help mucosal clearance are being used. N-acetylcysteine that solubilizes mucus glycoprotein, however, has not proved to be significantly effective. Recombinant human DNAse decreases the viscosity of sputum by degrading the concentrated amount of DNA in the sputum of CF patients. DNAse treatment has been beneficial in increasing airflow during short-term use, and has also prolonged the interval between episodes of pulmonary exacerbations.

CF patients are typically hospitalized somewhat regularly, often every 6 months depending on the severity of the case. Patients often have intravenous antibiotics through a PICC line, Central Line, or Port-a-Caths.

Cystic fibrosis can also lead to bronchiectasis. Bronchiectasis is an abnormal stretching and enlarging of the respiratory passages caused by mucus blockage. When the body is unable to get rid of mucus, mucus becomes stuck and accumulates in the airways. The blockage and accompanying infection cause inflammation, leading to the weakening and widening of the passages. The weakened passages can become scarred and deformed, allowing more mucus and bacteria to accumulate, resulting in a cycle of infection and blocked airways. Bronchiectasis is a disease that causes localized, irreversible dilatation of part of the bronchial tree. Involved bronchi are dilated, inflamed, and easily collapsible, resulting in airflow obstruction and impaired clearance of secretions. Bronchiectasis is associated with a wide range of disorders, but it usually results from necrotizing bacterial infections, such as infections caused by the *Staphylococcus* or *Klebsiella* species or *Bordatella pertussis*.

Bronchiectasis is one of the chronic obstructive pulmonary diseases (COPD) and it can be complicated by emphysema and bronchitis. The disease is commonly misdiagnosed as asthma or pneumonia. Bronchiectasis can develop at any age, begins most often in childhood, but symptoms may not be apparent until much later. Bronchiectasis can occur as part of a birth defect, such as primary ciliary dyskinesia or cystic fibrosis. About 50% of all cases of bronchiectasis in the U.S. result from cystic fibrosis. It can also develop after birth as a result of injury or other diseases, like tuberculosis, pneumonia and influenza.

Dilation of the bronchial walls results in airflow obstruction and impaired clearance of secretions because the dilated areas interrupt normal air pressure of the bronchial tubes, causing sputum to pool inside the dilated areas instead of being pushed upward. The pooled sputum provides an environment conducive to the growth of infectious pathogens, and these areas of the lungs are thus very vulnerable to infection. The more infections that the lungs experience, the more damaged the lung tissue and alveoli become. When this happens, the bronchial tubes become more inelastic and dilated, which creates a perpetual, destructive cycle within this disease.

There are three types of bronchiectasis, varying by level of severity. Fusiform (cylindrical) bronchiectasis (the most common type) refers to mildly inflamed bronchi that fail to taper distally. In varicose bronchiectasis, the bronchial walls appear beaded, because areas of dilation are mixed with areas of constriction. Saccular (cystic) bronchiectasis is characterized by severe, irreversible ballooning of the bronchi peripherally, with or without air-fluid levels. Chronic productive cough is prominent, occurring in up to 90% of patients with bronchiectasis. Sputum is produced on a daily basis in 76% of patients.

In addition to CF, other genetic causes or contributing factors to bronchiectasisis include Kartagener syndrome, Young's syndrome, alpha 1-antitrypsin deficiency, and Primary immunodeficiencies. Acquired bronchiectasis occurs more frequently, with one of the biggest causes being tuberculosis. A especially common cause of the disease in children is Acquired Immunodeficiency Syndrome, stemming from the human immunodeficiency virus. Other causes of bronchiectasis include respiratory infections, obstructions, inhalation and aspiration of ammonia, and other toxic gases, pulmonary aspiration, alcoholism, heroin use and allergies. Cigarette smoking may also contribute to bronchiectasis.

The diagnosis of bronchiectasis is based on the review of clinical history and characteristic patterns in high-resolution CT scan findings. Such patterns include "tree-in-bud" abnormalities and cysts with definable borders. Bronchiectasis may also be diagnosed without CT scan confirmation if clinical history clearly demonstrates frequent, respiratory infections, as well confirmation of an underlying problem via blood work and sputum culture samples.

Symptoms include coughing (worsened when lying down), shortness of breath, abnormal chest sounds, weakness, weight loss, and fatigue. With infections the mucus may be discolored, foul smelling and may contain blood. Symptom severity varies widely from patient to patient and occasionally, a patient is asymptomatic.

Treatment of bronchiectasis is aimed at controlling infections and bronchial secretions, relieving airway obstruction, and preventing complications. This includes prolonged usage of antibiotics to prevent detrimental infections, as well as eliminating accumulated fluid with postural drainage and chest physiotherapy. Surgery may also be used to treat localized bronchiectasis, removing obstructions that could cause progression of the disease.

Inhaled steroid therapy that is consistently adhered to can reduce sputum production and decrease airway constriction over a period of time will prevent progression of bronchiectasis. One commonly used therapy is beclometasone dipropionate, also used in asthma treatment. Use of inhalers such as Albuterol (Salbutamol), Fluticasone (Flovent/Flixotide) and Ipratropium (Atrovent) may help reduce likelihood of infection by clearing the airways and decreasing inflammation.

Mannitol dry inhalation powder, under the name Bronchitol, has been approved by the FDA for use in Cystic Fibrosis patients with Bronchiectasis. The original orphan drug indication approved in February 2005 allowed its use for the treatment of bronchiectasis. The original approval was based on the results of phase 2 clinical studies showing the product

to be safe, well-tolerated, and effective for stimulating mucus hydration/clearance, thereby improving quality of life in patients with chronic obstructive lung diseases like Bronchiectasis. Long-term studies are underway as of 2007 to ensure the safety and effectiveness of the treatment.

Bronchiectasis patients are often given antibiotics for infection and bronchodilator medicines to open passages. Sometimes antibiotics are prescribed for a long period to prevent recurring infections, especially in people who have cystic fibrosis. There are also physical therapy techniques to help clear mucus. Lung transplants are also an option for severe cases. Fatalities are uncommon but may result from massive hemorrhage. If lung infections are treated immediately, bronchiectasis is less likely to develop.

Pneumonia is an illness of the lungs and respiratory system in which the alveoli (microscopic air-filled sacs of the lung responsible for absorbing oxygen from the atmosphere) become inflamed and flooded with fluid. Pneumonia can result from a variety of causes, including infection with bacteria, viruses, fungi, or parasites, and chemical or physical injury to the lungs. Typical symptoms associated with pneumonia include cough, chest pain, fever, and difficulty in breathing. Diagnostic tools include x-rays and examination of the sputum.

Therefore, there is a need for therapies to treat pulmonary disorders, including CF, pulmonary infections, COPD, bronchiectasis and others. Additionally, there is a need to improve lung function in patients having such disorders.

SUMMARY OF THE INVENTION

The present invention relates in part to a method of treating a pulmonary disorder in a patient comprising administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein:

the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

In some embodiments, the treatment cycle is administered to the patient at least twice. In some embodiments, the administration period is 15 to 35 days, or 20 to 35 days. In other embodiments, the administration period is about 28 days. In some embodiments, the off period is 15 to 35 days, or 20 to 35 days. In other embodiments, the off period is about 28 days. In still other embodiments, the off period is of 25 to 75 days, 35 to 75 days, or 45 to 75 days. In other embodiments, the off period is about 56 days.

In some embodiments, the administration period is about 28 days and the off period is about 28 days, while in other embodiments, the administration period is about 28 days and the off period is about 56 days.

In some embodiments, the effective dose comprises 250 to 1,500 mg of amikacin, 250 to 1000 mg of amikacin, or about 280 to about 560 mg of amikacin. In other embodiments, the effective dose is about 280 or about 560 mg of amikacin.

In some embodiments, the pulmonary disorder is selected from the group consisting of chronic obstructive pulmonary disease, bronchiectasis, pulmonary infection, cystic fibrosis, alpha-1-antitrypsin enzyme deficiency and a combination thereof. In other embodiments, the pulmonary condition is a bacterial pulmonary infection, such as a *P. aeruginosa* infection. In some embodiments, the pulmonary condition is bronchiectasis.

In some embodiments, the patient has a serum C_{max} of amikacin of less than about 10 mcg/mL during the administration period. In other embodiments, the patient has a sputum C_{max} of amikacin of at least 1000 mcg per gram of sputum either during the administration, for at least 15 days after the administration.

In some embodiments, the patient has a reduction in $\log_{10}$ CFU of the bacterial infection in the lungs of at least 0.5 for at least 15 days after the administration period ends. In other embodiments, the reduction in the $\log_{10}$ CFU is at least 1.0.

In some embodiments, the patient experiences an improvement in lung function for at least 15 days after the administration period ends. For example, the patient may experience an increase in FEV_1, an increase in blood oxygen saturation, or both. In some embodiments, the patient has an FEV_1 that is increased by at least 5% over the FEV_1 prior to the treatment cycle. In other embodiments, FEV_1 is increased by 5 to 50%. In other embodiments, FEV_1 is increased by 25 to 500 mL over FEV_1 prior to the treatment cycle. In some embodiments, blood oxygen saturation is increased by at least 1% over oxygen saturation prior to the treatment cycle.

In some embodiments, the length of time to a pulmonary exacerbation is at least 20 days from the last day of administration. In other embodiments, the length of time to a rescue treatment is at least 25 days from the last day of the administration.

In some embodiments, the liposomal amikacin formulation comprises a lipid selected from the group consisting of egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG), egg phosphatidylinositol (EPI), egg phosphatidylserine (EPS), phosphatidylethanolamine (EPE), phosphatidic acid (EPA), soy phosphatidylcholine (SPC), soy phosphatidylglycerol (SPG), soy phosphatidylserine (SPS), soy phosphatidylinositol (SPI), soy phosphatidylethanolamine (SPE), soy phosphatidic acid (SPA), hydrogenated egg phosphatidylcholine (HEPC), hydrogenated egg phosphatidylglycerol (HEPG), hydrogenated egg phosphatidylinositol (HEPI), hydrogenated egg phosphatidylserine (HEPS), hydrogenated phosphatidylethanolamine (HEPE), hydrogenated phosphatidic acid (HEPA), hydrogenated soy phosphatidylcholine (HSPC), hydrogenated soy phosphatidylglycerol (HSPG), hydrogenated soy phosphatidylserine (HSPS), hydrogenated soy phosphatidylinositol (HSPI), hydrogenated soy phosphatidylethanolamine (HSPE), hydrogenated soy phosphatidic acid (HSPA), dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidylglycerol (DPPG), distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG), dioleylphosphatidyl-ethanolamine (DOPE), palmitoylstearoylphosphatidyl-choline (PSPC), palmitoylstearol-phosphatidylglycerol (PSPG), mono-oleoyl-phosphatidylethanolamine (MOPE), cholesterol, ergosterol, lanosterol, tocopherol, ammonium salts of fatty acids, ammonium salts of phospholipids, ammonium salts of glycerides, myristylamine, palmitylamine, laurylamine, stearylamine, dilauroyl ethylphosphocholine (DLEP), dimyristoyl ethylphosphocholine (DMEP), dipalmitoyl ethylphosphocholine (DPEP) and distearoyl ethylphosphocholine (DSEP), N-(2,3-di-(9-(Z)-octadecenyloxy)-prop-1-yl-N,N,N-trimethyl ammonium chloride (DOTMA), 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP), phosphatidyl-glycerols (PGs), phosphatidic acids (PAs), phosphatidylinositols (Pls), phosphatidyl serines (PSs), distearoylphosphatidylglycerol (DSPG), dimyristoylphosphatidylacid (DMPA), dipalmitoylphosphatidylacid (DPPA), distearoylphosphatidylacid (DSPA), dimyristoylphosphatidylinositol (DMPI), dipalmitoylphosphatidylinositol (DPPI), distearoylphospatidylinositol (DSPI), dimyristoylphosphatidylserine (DMPS), dipalmitoylphosphatidylserine (DPPS), distearoylphosphati-

dylserine (DSPS), and mixtures thereof. In other embodiments, the liposomal amikacin formulation comprises a phospholipid and a sterol, such as DPPC and cholesterol. In other embodiments, the liposomal amikacin formulation comprises DPPC and cholesterol in about a 2 to 1 ratio by weight. In some embodiments, the liposomal amikacin formulation has a lipid to drug ratio of about 0.5 to about 1.0, about 0.5 to 0.7, or about 0.6 by weight.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts mass distribution of Liposomal Amikacin nebulizate collected on impactor stages as a function of cutoff diameter. The three Liposomal Amikacin lots of Table 15 legend (designated as 1, 2, and 3) were used with the eFlow nebulizer and ACI system (solid symbols) or the LC Star nebulizer and NGI system (open symbols).

FIG. **2** depicts reduction in the Log_{10}CFU/Lungs of Rats after Inhalation of Liposomal Amikacin 75 mg/mL or Tobramycin. The symbols represent the Log_{10}CFU/lungs of each rat 18 days after the instillation of PA3064 in agar beads and 3 days after the last inhalation session of saline or one of the above antibiotics. The values at 2.0 Log_{10} CFU represent the lower limit of detection of bacteria in the lung in the method. The bar represents the mean of each group. The means and standard deviations and two-tail t-test results were calculated using Excel software by Microsoft.

FIG. **3** depicts reduction in the Log_{10}CFU/lungs of rats after Inhalation of Liposomal Amikacin and Tobramycin for 28 days. Equivalent doses of the above antibiotics were given by inhalation therapy but on different schedules. Tobramycin was given BID daily for a total of 104 min per day for 28 days. Liposomal Amikacin was given once daily for 80 min for 28 days (Q1D×28) as was saline. Liposomal Amikacin was also given once daily for 160 min every other day for 28 days (Q2D×14) or once daily for 160 min for 14 consecutive days (Q1DX14) then just observed until the rats were euthanized. The symbols represent the Log_{10}CFU/lungs of each rat 35 days after the instillation of *P. aeruginosa* 3064 in agar beads. The means and standard deviations and two-tail t-test were calculated using Excel software by Microsoft).

FIG. **4** depicts the study designs for Study 4, wherein patients received liposomal amikacin daily for 28 days, followed by monitoring for a 28 day period after the last day of administration.

FIG. **5** depicts a graph showing the percent increase in oxygen saturation over baseline in pediatric patients receiving a 280 mg dose of amikacin compared to a placebo.

FIG. **6** depicts a graph showing the oxygen saturation in pediatric patients receiving a 560 mg dose of amikacin compared to a placebo.

FIG. **7***a* depicts a graph of lung function change by age as measured by FEV1 in the placebo group. Data for placebo for both 280 and 560 mg amikacin arms of the study were pooled and divided by age. Also, data for ARIKACE® (liposomal amikacin) for 280 and 560 mg amikacin arms were pooled and divided by age.

FIG. **7***b* depicts the lung function change by age in the patients receiving inhaled liposomal amikacin.

FIG. **8** depicts a graph comparing the change in FEV1 (measured in mL) in the 560 mg and 280 mg amikacin groups, and the placebo group.

FIG. **9** depicts a graph of the change in FEV1 as a percent relative to baseline in the 560 mg amikacin, 280 mg amikacin, and placebo groups.

FIG. **10** depicts a graph of the Log CFU change in all patients.

FIG. **11** depicts a graph of the Log CFU change for mucoid strains.

FIG. **12** depicts the study design for the multi-cycle study of nebulized liposomal amikacin, wherein patients received liposomal amikacin for 6 cycles, each consisting of 28 days of treatment with 560 mg amikacin followed by 56 days off drug.

FIG. **13** depicts a graph of the change in FEV1 (measured in L) as a percent relative to baseline throughout the multi-cycle study.

FIG. **14** depicts a graph of the change in Log CFU over the 18 months of the multi-cycle study.

FIG. **15** depicts a graph of the median MIC_{90} over six cycles of amikacin therapy.

FIG. **16** is a diagram showing patient enrollment across the two Arikace® studies. In the European study, 77 patients were screened, and 66 were randomized. In the US study, 56 patients were screened, and 46 were randomized. *Across all randomized subjects, 7 subjects withdrew consent prior to dosing. Data shown is for the 105 subjects dosed at least once with Arikace® or placebo.

FIG. **17** shows the change in FEV1 (L) from Baseline through Day 56. Filled squares, solid line=Arikace® 560 mg, *p=0.033 at day 28, *p=0.003 at day 56 (compared with placebo). Filled triangles, solid line=Arikace® 280 mg, *p=0.009 at day 28 (compared with placebo). Filled squares, dashed line=Arikace® 140 mg. Filled diamonds, dashed line=Arikace® 70 mg. Filled circles, dashed line=placebo. The values above the abscissa are the number of subjects in each dose cohort providing data at each time point (70 mg/140 mg/280 mg/560 mg/placebo).

FIG. **18** shows the change in sputum density of *Pseudomonas aueruginosa* (log_{10} CFU) from Baseline through Day 35. Filled squares, solid line=Arikace® 560 mg, *p=0.007 at day 28, *p=0.021 at day 35. Filled triangles, solid line=Arikace® 280 mg. Filled squares, dashed line=Arikace® 140 mg. Filled diamonds, dashed line=Arikace® 70 mg. Filled circles, dashed line=placebo. The values above the abscissa are the number of subjects in each dose cohort providing data at each time point (70 mg/140 mg/280 mg/560 mg/placebo).

FIG. **19** shows the change in FEV_1 (% predicted) from Baseline through cycle 6 of Arikace®. Each cycle consisted of 28 days of once daily Arikace® (560 mg) followed by 56 days off study drug. Each colored box is a treatment cycle. Study days (every two weeks) are as shown on the abscissa, with the number of subjects at each time point as noted immediately above the study days. *p<0.0001 for FEV_1 at end of treatment following six cycles compared with baseline; **p=0.0001 for FEV_1 at 56 days post-treatment following six cycles compared with baseline.

FIG. **20** shows the change in sputum density of *Pseudomonas aueruginosa* (log_{10} CFU) from baseline through cycle 6 of Arikace®. Each set of three bars is the change in sputum *Pseudomonas aeruginosa* density compared with baseline (day 01 of cycle 01) for days 01, day 14, and day 28 of each respective Arikace® cycle. *P=0.003 for change in CFU across all of the Arikace® treatment cycles relative to baseline (cycle 01, day 01).

FIG. **21** shows the median amikacin MIC90 of *Pseudomonas* per Arikace® 560 mg treatment cycle. Each bar is the median amikacin MIC90 for *Pseudomonas* isolates at days 01 and 28 of each Arikace® treatment cycle. The numbers below the abscissa are the study days and number of subjects providing data at each time point.

DETAILED DESCRIPTION OF THE INVENTION

For convenience, before further description of the present invention, certain terms employed in the specification,

examples and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

The term "pulmonary disorder" refers to any disease, ailment, or other unhealthy condition related to the respiratory tract of a subject, particularly the lungs of a subject. Generally pulmonary distress results in difficulty of breathing.

The term "treating" is art-recognized and refers to curing as well as ameliorating at least one symptom of any condition or disease.

The term "prophylactic" or "therapeutic" treatment is art-recognized and refers to administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish, ameliorate or maintain the existing unwanted condition or side effects therefrom).

The terms "therapeutically effective dose" and "therapeutically effective amount" refer to that amount of a compound that results in prevention or amelioration of symptoms in a patient or a desired biological outcome, e.g., improved clinical signs, delayed onset of disease, reduced levels of bacteria, etc.

The term "FEV_1" is well known in the art as a measure of lung function, and refers to the forced expiratory volume in one second. The FEV_1 values used herein are measured in mLs, and also in terms of percent change from baseline, e.g., a change from pre-treatment values.

A "patient," "subject" or "host" to be treated by the subject method may mean either a human or non-human animal.

The term "mammal" is known in the art, and exemplary mammals include humans, primates, bovines, porcines, canines, felines, and rodents (e.g., mice and rats).

The term "bioavailable" is art-recognized and refers to a form of the subject invention that allows for it, or a portion of the amount administered, to be absorbed by, incorporated to, or otherwise physiologically available to a subject or patient to whom it is administered.

The term "pharmaceutically-acceptable salts" is art-recognized and refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds, including, for example, those contained in compositions of the present invention.

The term "pharmaceutically acceptable carrier" is art-recognized and refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition or component thereof from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the subject composition and its components and not injurious to the patient. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

II. Liposomal Amikacin

Liposomal amikacin formulations useful in the presently disclosed methods can be prepared as described, for example, in U.S. Publication No. 20060073198 or 20080089927, both of which are hereby incorporated by reference. Generally, amikacin is used in the form of a pharmaceutically acceptable salt, for example the sulfate salt of amikacin.

The lipids used in the compositions of the present invention can be synthetic, semisynthetic or naturally-occurring lipids, including phospholipids, tocopherols, steroids, fatty acids, glycoproteins such as albumin, anionic lipids and cationic lipids. The lipids may be anionic, cationic, or neutral. In one embodiment, the lipid formulation is substantially free of anionic lipids, substantially free of cationic lipids, or both. In one embodiment, the lipid formulation comprises only neutral lipids. In another embodiment, the lipid formulation is free of anionic lipids or cationic lipids or both. In another embodiment, the lipid is a phospholipid. Phospholipids include egg phosphatidyl choline (EPC), egg phosphatidylglycerol (EPG), egg phosphatidylinositol (EPI), egg phosphatidylserine (EPS), phosphatidylethanolamine (EPE), and egg phosphatidic acid (EPA); the soya counterparts, soy phosphatidyl choline (SPC); SPG, SPS, SPI, SPE, and SPA; the hydrogenated egg and soya counterparts (e.g., HEPC, HSPC), other phospholipids made up of ester linkages of fatty acids in the 2 and 3 of glycerol positions containing chains of 12 to 26 carbon atoms and different head groups in the 1 position of glycerol that include choline, glycerol, inositol, serine, ethanolamine, as well as the corresponding phosphatidic acids. The chains on these fatty acids can be saturated or unsaturated, and the phospholipid can be made up of fatty acids of different chain lengths and different degrees of unsaturation. In particular, the compositions of the formulations can include dipalmitoylphosphatidylcholine (DPPC), a major constituent of naturally-occurring lung surfactant as well as dioleoylphosphatidylcholine (DOPC). Other examples include dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) dipalmitoylphosphatidcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) distearoylphosphatidylcholine (DSPC) and distearoylphosphatidylglycerol (DSPG), dioleylphosphatidylethanolamine (DOPE) and mixed phospholipids like palmitoylstearoylphosphatidylcholine (PSPC) and palmitoylstearoylphosphatidylglycerol (PSPG), driacylglycerol, diacylglycerol, seranide, sphingosine, sphingomyelin and single acylated phospholipids like mono-oleoyl-phosphatidylethanol amine (MOPE).

The lipids used can include ammonium salts of fatty acids, phospholipids and glycerides, phosphatidylglycerols (PGs), phosphatidic acids (PAs), phosphotidylcholines (PCs), phosphatidylinositols (PIs) and the phosphatidylserines (PSs). The fatty acids include fatty acids of carbon chain lengths of 12 to 26 carbon atoms that are either saturated or unsaturated. Some specific examples include: myristylamine, palmitylamine, laurylamine and stearylamine, dilauroyl ethylphosphocholine (DLEP), dimyristoyl ethylphosphocholine (DMEP), dipalmitoyl ethylphosphocholine (DPEP) and distearoyl ethylphosphocholine (DSEP), N-(2,3-di-(9(Z)-octa-

decenyloxy)-prop-1-yl-N,N,N-trimethylammonium chloride (DOTMA) and 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP). Examples of PGs, PAs, PIs, PCs and PSs include DMPG, DPPG, DSPG, DMPA, DPPA, DSPA, DMPI, DPPI, DSPI, DMPS, DPPS and DSPS, DSPC, DPPG, DMPC, DOPC, egg PC.

In another embodiment, the liposome comprises a lipid selected from the group consisting of phosphatidyl cholines (PCs), phosphatidyl-glycerols (PGs), phosphatidic acids (PAs), phosphatidylinositols (Pls), and phosphatidyl serines (PSs).

In another embodiment, the lipid is selected from the group consisting of: egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG), egg phosphatidylinositol (EPI), egg phosphatidylserine (EPS), phosphatidylethanolamine (EPE), phosphatidic acid (EPA), soy phosphatidylcholine (SPC), soy phosphatidylglycerol (SPG), soy phosphatidylserine (SPS), soy phosphatidylinositol (SPI), soy phosphatidylethanolamine (SPE), soy phosphatidic acid (SPA), hydrogenated egg phosphatidyl choline (HEPC), hydrogenated egg phosphatidylglycerol (HEPG), hydrogenated egg phosphatidylinositol (HEPI), hydrogenated egg phosphatidylserine (REPS), hydrogenated phosphatidylethanolamine (HEPE), hydrogenated phosphatidic acid (HEPA), hydrogenated soy phosphatidylcholine (HSPC), hydrogenated soy phosphatidylglycerol (HSPG), hydrogenated soy phosphatidylserine (HSPS), hydrogenated soy phosphatidylinositol (HSPI), hydrogenated soy phosphatidylethanolamine (HSPE), hydrogenated soy phosphatidic acid (HSPA), dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidyl glycerol (DPPG), distearoylphosphatidylcholine (DSPC), distearoylphosphatidylglycerol (DSPG), dioleylphosphatidyl-ethanolamine (DOPE), palmitoylstearoylphosphatidyl-choline (PSPC), palmitoylstearolphosphatidylglycerol (PSPG), mono-oleoyl-phosphatidylethanolamine (MOPE), tocopherol, ammonium salts of fatty acids, ammonium salts of phospholipids, ammonium salts of glycerides, myristylamine, palmitylamine, laurylamine, stearylamine, dilauroyl ethylphosphocholine (DLEP), dimyristoyl ethylphosphocholine (DMEP), dipalmitoyl ethylphosphocholine (DPEP) and distearoyl ethylphosphocholine (DSEP), N-(2,3-di-(9-(Z)-octadecenyloxy)-prop-1-yl-N,N,N-trimethylammonium chloride (DOTMA), 1,2-bis (oleoyloxy)-3-(trimethylammonio)propane (DOTAP), distearoylphosphatidylglycerol (DSPG), dimyristoylphosphatidylacid (DMPA), dipalmitoylphosphatidylacid (DPPA), distearoylphosphatidylacid (DSPA), dimyristoylphosphatidylinositol (DMPI), dipalmitoylphosphatidylinositol (DPPI), distearoylphospatidylinositol (DSPI), dimyristoylphosphatidylserine (DMPS), dipalmitoylphosphatidylserine (DPPS), distearoylphosphatidylserine (DSPS), and mixtures thereof.

In another embodiment, the liposome comprises a phosphatidyl choline. The phosphatidyl choline may be unsaturated, such as DOPC or POPC, or saturated, such as DPPC. In another embodiment, the liposome does not include a sterol. In one embodiment, the liposome consists essentially of a phosphatidyl choline and a sterol. In another embodiment, the liposome consists essentially of DPPC and cholesterol.

Liposomes or lipid antiinfective formulations composed of phosphatidylcholines, such as DPPC, aid in the uptake by the cells in the lung such as the alveolar macrophages and helps to sustain release of the antiinfective agent in the lung (Gonzales-Rothi et al. (1991)). The negatively charged lipids such as the PGs, PAs, PSs and PIs, in addition to reducing particle aggregation, can play a role in the sustained release characteristics of the inhalation formulation as well as in the transport of the formulation across the lung (transcytosis) for systemic uptake.

While not being bound by any particular theory, it is believed that when the lipid comprises a neutral lipid, and does not comprise a negatively charged or positively charged phospholipid, the liposomal formulation has improved uptake by the lungs. For example, the liposome my have improved penetration into a biofilm or mucus layer when the lipid comprises only neutral lipids. Exemplary neutral lipids include the aforementioned phosphatidylcholines, such as DPPC and sterols, such as cholesterol.

IV. Methods of Treatment

The present invention is directed to methods of treating a pulmonary condition in a subject need thereof comprising administering to the subject and effective amount of any one of the aforementioned liposomal antibiotic formulations. In some embodiments, the pulmonary condition is a bacterial infection. In some embodiments, the method comprises administering to a patient in need thereof an effective amount of a liposomal amikacin formulation (also referred to herein as "liposomal amikacin") by inhalation daily. In some embodiments, the administration by inhalation comprises nebulizing the liposomal formulation.

In some embodiments, the liposomal amikacin formulation is administered daily for a period of time, followed by second period of time (an "off" period) wherein no liposomal formulation is administered. For example, in some embodiments, the method of treating a pulmonary disorder comprises administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein:

the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

In some embodiments, the aforementioned treatment cycle is administered to the patient at least twice. In other embodiments, the treatment cycle may be administered 3, 4, 5, 6, or more times.

During the administration period, liposomal amikacin is administered daily. In some embodiments, liposomal amikacin can be administered every other day or every third day during the administration period. As explained above, the administration period can be 15 to 75 days. In some embodiments, the administration period is 15 to 35 days, or 20 to 35 days. In other embodiments, the administration period is 20 to 30 days, 25 to 35 days or 25 to 30 days. In other embodiments, the administration period is about 25, 26, 27, 28, 29 or 30 days. In another embodiment, the administration period is about 28 days.

During the off period the liposomal amikacin formulation is not administered to the patient. In some embodiments, the off period is 15 days or longer, for example, 15 to 75 days, 15 to 35 days, or 20 to 35 days. In other embodiments, the off period is 20 to 30 days, 25 to 35 days or 25 to 30 days. In other embodiments, the off period is about 25, 26, 27, 28, 29 or 30 days. In other embodiments, the off period is about 28 days, while in still other embodiments, the off period is at least 29 days.

In some embodiments, the off period is of 25 to 75 days, 35 to 75 days, or 45 to 75 days. In other embodiments, the off period is 50 to 75 days, 50 to 70 days, 50 to 65 days or 50 to 60 days. In other embodiments, the off period is about 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 days, while in other embodiments, the off period is about 56 days.

In some embodiments, the administration period is about 28 days and the off period is about 28 days, while in other embodiments, the administration period is about 28 days and the off period is about 56 days.

In some embodiments, the effective dose comprises 250 to 1,500 mg of amikacin, 250 to 1000 mg of amikacin, 250 to 750 mg of amikacin, or 250 to 700 mg amikacin each day of the administration period. In other embodiments, the effective dose is about 280 to about 560 mg of amikacin. In other embodiments, the effective dose is about 230 mg to about 330 mg, or about 510 mg to about 610 mg. In other embodiments, the effective dose of amikacin is about 100, 150, 200, 250, 300, 250, 400, 450, 500, 550, 600, 650, 700 or 750 mg of amikacin daily. In other embodiments, the effective dose is about 280 or about 560 mg of amikacin.

In some embodiments, the administration period is about 28 days, and the dose is about 280 to about 560 mg of amikacin. In other embodiments, the administration period is about 28 days, the off period is about 28 days, and the dose is about 280 to about 560 mg. In other embodiments, the administration period is about 28 days, the off period is about 56 days, and the dose is about 280 o about 560 mg.

In some embodiments, the pulmonary disorder is selected from the group consisting of chronic obstructive pulmonary disease, bronchiectasis, pulmonary infection, cystic fibrosis, alpha-1-antitrypsin enzyme deficiency and a combination thereof. In some embodiments, the pulmonary condition is cystic fibrosis. In other embodiments, the pulmonary condition is a bacterial pulmonary infection, *Pseudomonas* (e.g., *P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens*, and *P. acidovorans*), staphylococcal, Methicillin-resistant *Staphylococcus aureus* (MRSA), streptococcal (including by *Streptococcus pneumoniae*), *Escherichia coli, Klebsiella, Enterobacter, Serratia, Haemophilus, Yersinia pesos, Burkholderia pseudomallei, B. cepacia, B. gladioli, B. multivorans, B. vietnamiensis, Mycobacterium tuberculosis, M. avium* complex (MAC)(*M. avium* and *M. intracellulare*), *M kansasii, M. xenopi, M marinum, M. ulcerans*, or *M. fortuitum complex* (*M. fortuitum* and *M. chelonae*) infections. In some embodiments, the infection is a *P. aeruginosa* infection, while in other embodiments, the infection is a non-tuberculous mycobacterial infection. The pulmonary infection may or may not be associated with cystic fibrosis. Thus, in some embodiments, the pulmonary condition is both cystic fibrosis and a pulmonary infections such as *P. aeruginosa*. In other embodiments, the pulmonary conditions is bronchiectasis. The bronchiectasis may or may not be associated with cystic fibrosis.

In one embodiment, infections caused by the following bacteria are treatable with the treatment regimens and formulations provided herein: *Pseudomonas* (e.g., *P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens*, and *P. acidovorans*), *Burkholderia* (e.g., *B. pseudomallei, B. cepacia, B. cepacia complex, B. dolosa, B. fungorum, B. gladioli, B. multivorans, B. vietnamiensis, B. pseudomallei, B. ambifarict, B. andropogonis, B. anthina, B. brasilensis, B. caledonica, B. caribensis, B. caryophylli*), *Staphylococcus* (e.g., *S. aureus, S. auricularis, S. carnosus, S. epidermidis, S. lugdunensis*), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus* (e.g., *Streptococcus pneumoniae*), *Escherichia coli, Enterobacter, Serratia, Haemophilus, Yersinia pestis, Mycobacterium* (e.g., nontuberculous mycobacterium, *M. abscessus, M. chelonae, M. bolletii, M. tuberculosis, M. avium complex* (MAC) (*M. avium* and *M. intracellulare*), *M. kansasii, M. xenopi, M. marinum, M. ulcerans*, or *M. fortuitum complex* (*M. fortuitum* and *M. chelonae*)).

The present method provides advantageous levels of amikacin at the site of the pulmonary disorder, while limiting systemic exposure to the drug, and also provides a sustained benefit to the subject for surprisingly extended periods of time. While not being bound by any particular theory, it is believed that administration of liposomal amikacin in accordance the with methods described herein results a "depot" effect in the lungs of the subject. Specifically, it is believed that the liposome particles are small enough and contain an appropriate lipid formulation to penetrate and diffuse through CF sputum and into the bacterial biofilm. The liposomes shield the entrapped cationic amikacin in neutral liposomes to minimize electrostatic interaction with the negatively charged sputum/biofilm, which would otherwise reduce its bioavailability. In addition, there are *P. aeruginosa* derived virulence factors (rhamnolipids) (Davey et al. 2003), which release amikacin the liposomes. Therefore, it is hypothesized that relatively high concentrations of drug can be delivered locally to the bacterial macro-colony environment.

Additionally, it is believed that inhalation of liposomal amikacin leads to a dose dependent recruitment of macrophages as an adaptive response to inhalation of drug/lipid formulation. The presence of alveolar macrophages (which have been shown to be functionally normal in liposomal amikacin treated rats) may be particularly beneficial in CF patients. CF patients are known to have reduced number of macrophages in their lungs and possibly with poor functionality, which may contribute to the chronicity of *P. aeruginosa* lung infection, and to the higher prevalence of non-tuberculous mycobacterial infection in this population. The dose dependent recruitment of macrophages may also contribute to the sustained effects observed using the methods of the present invention. Specifically, the macrophages in the lung may take up liposomal amikacin, and then remain in the lung for a period of time, followed by release of the liposomal amikacin by the macrophages. A clinical study (described in the exemplification below) of liposomal amikacin in CF patients chronically infected with *P. aeruginosa* has demonstrated safety, tolerability and dose dependent improvement in lung function and respiratory symptoms; and reduction of sputum bacterial density at the end of 28 days of treatment. This improvement in lung function was sustained for at least 28 days after completion of treatment (Day 56) with a 560 mg dose of liposomal amikacin, indicating a sustained treatment effect.

The present method thus provides, in some embodiments, advantageous levels of amikacin in the blood and in the sputum. For example, the methods provides relatively low systemic exposure to amikacin, while providing high, sustained levels of amikacin at the site of the pulmonary condition. For example, in some embodiments, the patient has a serum C_{max} of amikacin of less than about 25 mcg/mL during the administration period. In other embodiments, the serum C_{max} is less than 20, 15, 10, 5 or 2 mcg/mL during the administration period.

In some embodiments, the patient has a sputum C_{max} of amikacin of at least about 500 mcg per gram of sputum either during the administration, or for a sustained period of time, such as at least 15 days, after the administration. In other embodiments, the sputum C_{max} of amikacin is at least 750, 1000, 1500, 2000, 2500, 3000 or 3500 mcg per gram of sputum.

When the pulmonary disorder includes a pulmonary infection, the present invention also provides a reduction in the colony forming units of the bacteria in the lung for a sustained period of time. For example, the CFU's are reduced compared to a baseline value. In some embodiments, the patient has a reduction in $\log_{10}$ CFU of the bacterial infection in the lungs of at least about 0.5 for at least 15 days after the administration period ends. In other embodiments, the reduction in the

log_{10} CFU is at least by 1.0, 1.5, 2.0 or 2.5. *Pseudomonas* infections, in particular, can form large colonies, known as "mucoid" *Pseudomonas*, particularly in patients with cystic fibrosis. In some embodiments, the CFU's are reduced as described above in a mucoid strain of a *Pseudomonas* infection.

In some embodiments, the patient experiences an improvement in lung function for at least 15 days after the administration period ends. For example, the patient may experience an increase in the forced expiratory volume in one second (FEV_1), an increase in blood oxygen saturation, or both. In some embodiments, the patient has an FEV_1 that is increased by at least 5% or at least 10% over the FEV_1 prior to the treatment cycle. In other embodiments, FEV_1 is increased by 5 to 50%, 5 to 25%, or 5 to 20%. In other embodiments, FEV_1 is increased by 5 to 15% or 5 to 10%. In other embodiments, FEV_1 is increased by 10 to 50%, 10 to 40%, 10 to 30% or 10 20%. FEV_1 is frequently measured in mL. Accordingly, in some embodiments, FEV_1 is increased by at least 25 mL when compared to FEV_1 prior to the treatment. In some embodiments, FEV_1 is increased by 25 to 500 mL, 25 to 400, 25 to 300 or 25 to mL. In other embodiments, FEV_1 is increased by 50 to 500 mL, 50 to 400 mL, 50 to 300 mL, 50 to 200 mL or 50 to 100 mL.

In some embodiments, blood oxygen saturation is increased in the subject compared to the blood oxygen saturation levels prior to the administration. In some embodiments, blood oxygen saturation is increased by at least 1% or by at least 2% for at least 15 days after the administration period. In other embodiments, the blood oxygen saturation levels are increased by about 1 to 50%, 1 to 25%, 1 to 20%, 1 to 15%, 1 to 10% or 1 to 5%. In other embodiments, the blood oxygen saturation levels are increased by about 2 to 10% or 2 to 5%.

The aforementioned sustained periods of time may be at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 or 75 days after the administration period. In other embodiments, the sustained period of time is at least 28, 35, 42, 48 or 56 days after the administration period. In other embodiments, sustained period of 15 to 75 days, 15 to 35 days, or 20 to 35 days. In other embodiments, the sustained period of time is 20 to 30 days, 25 to 35 days or 25 to 30 days. In other embodiments, the sustained period of time is about 25, about 26, about 27, about 28, about 29 or about 30 days, or about 28 days, or at least 29 days. In other embodiments, the sustained period of time during is 25 to 75 days, 35 to 75 days, or 45 to 75 days. In other embodiments, the sustained period is 50 to 75 days, 50 to 70 days, 50 to 65 days or 50 to 60 days. In other embodiments, the sustain period is about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59 or about 60 days, while in other embodiments, the sustained period is about 56 days.

In some embodiments, the aforementioned methods advantageously provide a reduced incidence of pulmonary exacerbations in the patient. The method also advantageously increases the length of time to pulmonary exacerbation. For example, in some embodiments, the length of time to pulmonary exacerbation is at least about 20 days. In other embodiments, the length of time is 20 to 100 days. In other embodiments, the length of time is 25 to 100 days, 30 to 100 days, 35 to 100 days or 40 to 100 days. In other embodiments, the length of time is 25 to 75 days, 30 to 75 days, 35 to 75 days or 40 to 75 days. In other embodiments, the length of time is 30 to 60 days.

In some embodiments, the incidence of rescue treatment is reduced. In other embodiments, the length of time to rescue treatment is reduced, for example when the patient has a pulmonary infection, the time to anti-infective rescue treatment is reduced. In some embodiments, the length of time is 20 to 100 days. In other embodiments, the length of time is 25 to 100 days, 30 to 100 days, 35 to 100 days or 40 to 100 days. In other embodiments, the length of time is 25 to 75 days, 30 to 75 days, 35 to 75 days or 40 to 75 days. In other embodiments, the length of time is 30 to 60 days.

In some embodiments, the liposomal amikacin formulation used in the aforementioned methods comprises amikacin and any of the lipids described above. In some embodiments, the liposomal amikacin formulation comprises a phospholipid and a sterol, such as DPPC and cholesterol. In other embodiments, the liposomal amikacin formulation comprises DPPC and cholesterol in about a 2 to 1 ratio by weight. In some embodiments, the liposomal amikacin formulation has a lipid to drug ratio of about 0.5 to about 1.0, about 0.5 to 0.7, or about 0.6 by weight. In other embodiments, the liposomal amikacin formulation has a lipid to drug ratio of about 0.3 to about 1.0 by weight, while in other embodiments, the lipid to drug ratio is about 0.5 to 0.7 by weight, or about 0.65 by weight. The liposomes in the formulation may have a amend diameter of 100 to 100 nm, 100 to 500 nm, 200 to 500 nm, or about 300 nm. In some embodiments, the total concentration of amikacin in the liposomal amikacin formulation is about 20 to 100 mg/mL, 20 to 90 mg, mL, 30 to 90 mg/mL, 30 to 80 mg/mL, or 40 to 80 mg/mL. In other embodiments, the concentration is about 30, 40, 50, 60, 70, 80 or 90 mg/mL.

In some embodiments, aforementioned method comprises:

administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein:

the treatment cycle comprises an administration period of about 28 days, followed by an off period of about 28 days;

the effective dose comprises about 280 to about 560 mg of amikacin daily during the administration period; and

the liposomal amikacin formulation comprises DPPC and cholesterol in about a 2:1 ratio, and a lipid to amikacin ratio of about 0.5 to about 0.7.

In other embodiments, the method comprises:

administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein:

the treatment cycle comprises an administration period of about 28 days, followed by an off period of about 56 days;

the effective dose comprises about 280 to about 560 mg of amikacin daily during the administration period; and

the liposomal amikacin formulation comprises DPPC and cholesterol in about a 2:1 ratio, and a lipid to amikacin ratio of about 0.5 to about 0.7.

In other embodiments, the present invention relates to a method of providing a sustained treatment effect in a subject comprising: administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein: the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

In another embodiment, the present invention relates to a method of improving oxygen saturation levels in a patient with a pulmonary condition comprising: administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein: the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

In another embodiment, the present invention relates to a method of improving FEV_1 in a patient with a pulmonary condition comprising: administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein: the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period.

In another embodiment, the present invention relates to a method of reducing bacterial density in the lung or sputum of a patient with a bacterial pulmonary infection comprising: administering to the patient an effective dose of a nebulized liposomal amikacin formulation for at least one treatment cycle, wherein: the treatment cycle comprises an administration period of 15 to 75 days, followed by an off period of 15 to 75 days; and the effective dose comprises 100 to 2500 mg of amikacin daily during the administration period, and wherein the bacterial density remains reduced for at least 15 days after the last day of the administration.

EXAMPLES

Introduction to Materials and Methods

Lipid based or liposomal aminoglycoside, such as amikacin, formulations for inhalation are sustained-release formulations of aminoglycosides encapsulated inside nanoscale liposomal carriers designed for administration via inhalation. Sustained-release targeting of high concentrations of amikacin in the lungs and biofilm penetration properties of these formulations have several advantages over inhalation of the "free" antibiotic, e.g., inhaled tobramycin. Amikacin can be encapsulated in liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and cholesterol, at a targeted lipid-to-drug ratio of about 0.6-0.7:1 (w/w). An example of a ~70 mg/mL liposomal amikacin formulation useful in the aforementioned methods is presented below:

Component	Concentration
Amikacin[1]	~70 mg/mL
Dipalmitoylphosphatidylcholine (DPPC)	~30 mg/mL
Cholesterol	~15 mg/mL
1.5% NaCl	QS

[1]Added to the formulation as Amikacin sulfate, USP.

These formulations can be prepared according to the methods described in U.S. Publication No. 20060073198 or 20080089927, both of which are hereby incorporated by reference.

These formulations have several advantages in treating pulmonary conditions, for example, CF subjects with chronic infection caused by *P. aeruginosa*, including:

1. The ability to attain a prolonged antibiotic effect of amikacin in the lung by achieving high concentrations and a prolonged half life due to sustained release.
2. The ability to target and increase the effective concentration of amikacin in the lung with low systemic levels of the aminoglycoside.
3. The potential to better target bacteria growing in a biofilm as a result of unique properties of lipid based or liposomal aminoglycosides.
4. Additional release of the drug at the site of infection in the lungs of CF patients, due to targeted action of secreted phospholipase C and rhamnolipids from bacteria and/or phospholipase A2 or defensins from activated polymorphonuclear leukocytes.
5. Amikacin is a semisynthetic aminoglycoside with a unique resistance to aminoglycoside inactivating enzymes. Consequently, some *P. aeruginosa* strains which are resistant to tobramycin will likely remain susceptible to amikacin.
6. Amikacin has less binding affinity than other aminoglycosides for megalin, the transporter responsible for renal cortical aminoglycoside accumulation, and thus inherently has a lower potential for nephrotoxicity.
7. The increase in both the half life, and the area under the concentration curve (AUC) of lipid based or liposomal amikacin, along with biofilm penetration should allow for less frequent administration, enhanced bactericidal activity and reduced potential for selection of resistant organisms.

Preclinical pharmacokinetics have demonstrated that the AUC (0-48 hr) of amikacin in the lungs of rats that received a 60 mg/kg dose aerosol of Liposomal Amikacin was five-fold higher than the AUC of tobramycin in the lungs of rats that received an equal dose of tobramycin by inhalation. Generally, 10% of the administered antibiotic is deposited in the lungs for rats. Conversely, the AUC of drug in the kidneys of rats that received an equal dose of tobramycin was significantly higher than the kidney AUC of rats that received aerosols of Liposomal Amikacin. Additionally, data from 30-day inhalation toxicology studies in rats and dogs suggest that there will be no safety pharmacology issues with inhaled Liposomal Amikacin.

In 14 days rat model studies of *pseudomonas* infection, it was noted that 60 mg/kg of Liposomal Amikacin (75 mg/mL) administered every other day for 14 days (Q2D×7), which effectively delivered half the cumulative dose of aminoglycoside than the other groups, was as effective as 60 mg/kg of Liposomal Amikacin given once per day, and Tobramycin given twice per day daily for 14 days. With 28 day dosing in this model, there were equivalent reductions in CFUs in animals receiving Liposomal Amikacin dosed daily at ~60 mg/kg or dosed every other day at ~120 mg/kg. Liposomal Amikacin administered at 120 mg/kg once a day for 14 days was as effective as Tobramycin 60 mg/kg/day (administered twice a day) for 28 days, which suggests a higher AUC and possibly a prolonged post-antibiotic effect with Liposomal Amikacin at 120 mg/kg dosed once per day (see Example 5).

The administration of Liposomal Amikacin via inhalation in the animal model resulted in increased lung (AUC) above the MIC of the bacteria, and demonstrated sustained therapeutic effect, with a reduced frequency, and duration of dosing as compared to Tobramycin. Importantly, the preclinical data for Liposomal Amikacin appear supportive of the hypothesis that this specific formulation may be advantageous over other inhalation products that are hindered by a rapid clearance from lung tissue, necessitating frequent dosing (Geller, Pitlick et al. 2002), which poses a burden for patients and might limit patient compliance.

Additionally, clinical experience demonstrated that nebulized Liposomal Amikacin 50 mg/mL administered as 500 mg once per day for 14 days is well tolerated, and elicits a clinically relevant effect on pulmonary function and decrease in *P. aeruginosa* density in CF patients. Also, evaluation of the PK data indicates the systemic exposure to Liposomal Amikacin, even at the 500 mg dose, is very low. By either Cmax or AUC or mg of aminoglycoside which is recovered in the urine, the observed systemic exposure to amikacin, associated with Liposomal Amikacin, given by inhalation is approximately ⅕ to ¼ the exposure seen with 600 mg/d of

TOBI and is less than ½00 compared to normal parenteral doses of amikacin. The data further indicate high levels of amikacin are achieved in the sputum. Median AUC values for sputum were 290 and 980 fold greater than the median AUC values for serum on day 1 and day 14 respectively.

Inhaled liposomal amikacin maintains prolonged targeted lung exposures and enhance the uptake of drug to the site of infection. Using data from a human clinical Phase 1b/2a study in which CF patients who were chronically infected with *P. aeruginosa* received multiple doses of Liposomal Amikacin 50 mg/ml, the objectives of the analyses described herein were three-fold: (1) to use population pharmacokinetic (PK) modeling to characterize amikacin systemic exposure, including approximate systemic bioavailability; (2) to characterize the disposition of liposomal amikacin in sputum; and 3) to characterize the pharmacokinetic-pharmacodynamic (PK-PD) relationship between change in forced expiratory volume in one second (FEV_1), change in percent predicted forced expiratory volume in one second (FEV_1% predicted), forced expired flow between 25-75% of forced vital capacity ($FEF_{25-75\%}$), and forced vital capacity (FVC), in *P. aeruginosa* colony forming units (CFU) relative to baseline at Days 7 and 14, and amikacin exposure.

Preclinical Studies with Liposomal Amikacin

Several preclinical studies were conducted with the 20 and 50 mg/mL formulations. Anti-*pseudomonas* activity of Liposomal Amikacin in in vitro and in vivo models was demonstrated. Additionally, studies confirmed that virulence factors secreted by *Pseudomonas* facilitate the further release of amikacin from the liposomes, and characterized the deposition and sustained release of amikacin in the lungs of rats, and dogs. The safety of a 30 day administration of Liposomal Amikacin in two species was also established.

Nonclinical pharmacokinetics have demonstrated that the AUC (0-48 hr) of amikacin in the lungs of rats that received a 60 mg/kg dose of Liposomal Amikacin via nebulization, was five-fold higher than the AUC of tobramycin in the lungs of rats that received an equal dose of tobramycin by inhalation. High levels of amikacin were sustained in the lung (>250 μg/mL through 150 hr), suggesting a depot effect. In contrast, lung levels of tobramycin were undetectable within 6 hours of cessation of administration. Conversely, the AUC of drug in the kidneys of rats that received an equal dose of tobramycin was significantly higher than the AUC of rats that received aerosols of Liposomal Amikacin. There were no significant differences in the AUC of aminoglycosides in the serum and urine of the animals; serum levels were undetectable after 24 hr. This profile supports the intended sustained release and depot effect of amikacin in the lung following administration of nebulized Liposomal Amikacin, potentially representing an enhanced efficacy profile. These data for Liposomal Amikacin appear supportive of the hypothesis that this specific formulation may be advantageous over other inhalation products that are hindered by a rapid clearance from lung tissue, necessitating frequent dosing (Geller, Pitlick et al. 2002), and placing a burden on patients. Additionally, toxicokinetic data from 30-day inhalation GLP toxicology studies in rats and dogs showed that there is a 15 fold increase in lung deposition of amikacin dogs as compared to the free amikacin treated group, with comparable plasma and urine levels, indicating high lung concentrations with low systemic exposure.

The pharmacodynamic effect of Liposomal Amikacin was evaluated in vivo in a rat model of chronic pulmonary infection with *Pseudomonas* (Cash, Woods et al. 1979). In a 14 days *Pseudomonas* infection model, 60 mg/kg of Liposomal Amikacin (75 mg/mL) was administered every other day for 14 days (Q2D×7). This regimen was as effective as 60 mg/kg of Liposomal Amikacin (given once per day for 14 days), and tobramycin (given twice per day for 14 days). When dosing was extended to 28 days, there were equivalent reductions in CFUs for animals receiving Liposomal Amikacin dosed daily at ~60 mg/kg or dosed every other day at ~120 mg/kg. Also, in this experiment, Liposomal Amikacin administered at 120 mg/kg once a day for 14 days was as effective as tobramycin 60 mg/kg/day (administered twice a day) for 28 days. This indicated a higher AUC and a prolonged post-antibiotic effect with Liposomal Amikacin at 120 mg/kg dosed once per day. The preclinical pharmacodynamic data were thus consistent with a sustained antimicrobial benefit enhanced by the site-specific delivery of drug to the lungs via inhalation.

Thus, administration of Liposomal Amikacin via inhalation resulted in increased lung concentrations (AUC) several fold above the MIC of the bacteria, with the potential to provide a sustained therapeutic effect with a reduced frequency and duration of dosing, particularly as compared to Tobramycin.

Example 1

Phase 1b/2a Study

Data used for this population PK analysis were obtained from two human clinical Phase 1b/2a studies in which CF patients, chronically infected with *P. aeruginosa*, were administered a total of 500 mg of Liposomal Amikacin daily (in two 20 minute sessions with a 5 minute rest period in between) for 14 days.

Amikacin serum samples were obtained pre-dose, and 1, 2, 4, 6, 8, 12 and 24 hours post-dose on Days 1 and 14, while urine samples were collected over 6 hour intervals on Day 1 and Day 14 for a period of 24 hours. Sputum samples were also collected on Day 1 and Day 14, soon after the dose was administered, between 4 and 6 hours after dosing and prior to dose administration on the following day, as well as on Days 14, 21, and 28. Serum, sputum and urine samples were assayed for amikacin using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).

Pulmonary function tests (PFT) were carried out during screening from Day −14 to 0) and at baseline (i.e., prior to dose administration on Day 1) and on Day 1, 7, 14, 21, 28, 35, and 42. Sputum samples for microbiology were also collected at baseline and on each of these days. Additional PFTs were carried out 1.5 hours and 3 hours post-dose on Day 1 and Day 14.

Pharmacokinetic Analysis

The data were fit by candidate PK models, using Monte Carlo Parametric Expectation Maximization (MC-PEM), as is implemented in S-ADAPT 1.53, initially fitting the plasma concentrations, then co-modeling the serum and urine data. Model discrimination was based on the fit of the data and change in objective function. The 24 hour area under the curve (AUC) at steady state for serum amikacin values were calculated using the post-hoc parameter estimates from the final population PK model. Covariate relationships between patient demographics and individual post-hoc parameters were assessed first graphically, then by means of statistical models created using SYSTAT® 11 (SYSTAT Software, Inc., Richmond, Calif.). Sputum AUC values from 0 to 24 hours on Day 1 and Day 14 were obtained using the linear trapezoidal rule.

Dependent variables for the PK-PD analysis included the change in PFT values for FEV_1, FEV_1% predicted, FEF_{25-75}% and FVC, on Day 7 and 14 relative to baseline (prior to dose administration on Day 1) and the change in

log_{10} CFU on each of these days relative to baseline. Independent variables evaluated included the ratio of the average 24 hour AUC for serum and sputum to the baseline minimum inhibitory concentration (MIC), AUC:MIC ratio for *P. aeruginosa*. The average 24 hour serum and sputum AUC was computed by taking the average of the Day 1 and Day 14 AUC values.

Using a one-sample t-test, the statistical significance of mean changes from baseline for each of the above-described dependent variables was assessed. Using Spearman's rank correlation (r_s), the direction and strength of the relationship between each of the dependent variables and AUC:MIC ratio for serum and sputum was assessed. The direction and strength of the relationship between change in each of the PFT values from baseline and change in log_{10} CFU from baseline were also assessed.

Results

A total of 24 patients completed the two studies with 13 patients from Study 1 and 11 patients from Study 2. The median (min, max) age of all the patients was 23.7 (14, 38) years with a median (range) creatinine clearance (CrCL) at baseline of 126 (76.8, 173) mLmin/1.73 m^2.

The most robust fit to the serum concentration data was obtained using a two-compartment model (one absorption site, the lung, and the central compartment) with zero-order drug input into the lungs, a first-order process from lungs to the central compartment and linear elimination. Allowing inter-occasional variation on apparent total clearance (CLt/F) and apparent central volume of distribution (Vc/F) between Day 1 and Day 14 improved the objective function statistically. Urine data was modeled by fitting the amounts of amikacin recovered in the collection intervals, as a function of serum concentrations and renal clearance (CLr). Table 1 is a summary of the fitted PK parameter values.

TABLE 1

Structural population pharmacokinetic model for liposomal amikacin for inhalation with inter-occasional variability - Parameter estimates and standard errors.

	Population mean		Inter-individual variability (% CV)	
Parameter	Final estimate	% SE	Final estimate	% SE
CLt/F Day 1 (L/hr)	68.4	10.3	48.7	29.9
Vc/F Day 1 (L)	286	12.3	59.0	29.7
ka (hr^{-1})	3.34	32.5	99.8	50.5
CLr (L/hr)	3.40	15.4	63.9	36.7
CLt/F Day 14 (L/hr)	45.2	8.01	37.1	30.7
Vc/F Day 14 (L)	250	8.51	27.0	30.8
$SDint_{serum}$	0.05	6.02		
$SDS1P_{Urine}$	0.70	9.16		
$SDint_{Urine}$	0.03			

Minimum value of the objective function = −258.6

The goodness of fit for observed versus Bayesian post-hoc individual fitted serum concentration data was excellent, with an overall r2 of 0.98.

The AUC values for the serum and sputum data are shown in Tables 2 and 3, respectively. Median AUC values for sputum were 286 and 978 fold greater than the median AUC values for serum on Day 1 and Day 14, respectively. As evidenced by the higher CV % values, greater variability was evident in sputum (117% on Day 1 and 91.2% on Day 14) compared to serum AUC (51.9% on Day 1 and 42.4% on Day 14) values.

TABLE 2

Summary of serum AUC values[1] - All patients

Study Day	N	Mean	SD	Min	Median	Max
Day 1	24	8.27	4.29	3.67	6.88	20.1
Day 14	24	12.0	5.08	5.65	10.8	30.1

[1]AUC values in mcg/mL · hr

TABLE 3

Summary of sputum AUC values[1] - All patients

Study Day	N	Mean	SD	Min	Median	Max
Day 1	20	3830	4500	78.70	1970	17200
Day 14	19	12500	11400	1740	10578	50000

[1]AUC values are in mcg/mL · hr

Serum (r^2=0.98) and urine (r^2=0.38) concentrations were well and modestly fit by model, respectively. On Day 7, 14 and 21, the observed change for FEF_{25-75}% was 0.49 (p<0.001), 0.42 (p=0.02) and 0.34 L/sec (p=0.04), respectively. On Day 7 and 14, the observed change for FEV_1 was 0.24 (p=0.002) and 0.13 L (p=0.10), respectively, and was 7.49 (p<0.001) and 4.38 L/sec (p=0.03) for FEV_1% predicted. Significant relationships (p≤0.05) between log_{10} CFU and serum AUC:MIC ratio, and between changes in log_{10} CFU and FEV_1, FEV_1% predicted and FVC were identified.

Baseline and Day 14 PFT data were available for all 24 patients and for PFTs carried out on Day 7 and 21, such data were available for 23 patients. Microbiology data were available for all 24 patients. Since MIC values collected prior to dosing on Day 1 for Study 2 were not reported, the screening MIC values as well as CFU counts were used as baseline values.

Using a one-sample t-test, the statistical significance of mean changes from baseline for each of the above-described dependent variables was assessed. Using Spearman's rank correlation (r_s), the direction and strength of the relationship between each of the dependent variables and AUC:MIC ratio for serum and sputum was assessed.

Mean changes in PFT values on Day 7 relative to baseline were statistically significant for all PFT endpoints. Mean changes in FEV_1% predicted and $FEF_{25.75\%}$ on Day 14 relative to baseline were also statistically significant (p=0.029 and p=0.016, respectively). By Day 21, mean change in $FEF_{25-75\%}$ relative to baseline was the single PFT that remained statistically significant (p=0.036). Regardless of the study day considered, mean change in log_{10} CFU from baseline was not statistically significant.

As shown in Table 4, correlations between change in PFT values from baseline and either sputum or serum AUC:MIC ratio were not statistically significant, regardless of whether changes on Day 7 or 14 were evaluated. As shown in Table 5, the correlation between change in log_{10} CFU from baseline and serum AUC:MIC ratio was statistically significant for both Day 7 or 14. Increasing serum AUC:MIC ratios were associated with larger decreases in log_{10} CFU on Day 7 (r_s=−0.46, p=0.048) and 14 (r_s=−0.45, p=0.048) relative to baseline.

Correlations between change in both PFT value and log_{10} CFU on Day 7 and 14 relative to baseline were statistically significant for FEV_1, FEV_1% predicted, and FVC (p<0.05).

TABLE 4

Relationship between change in pulmonary function test values from baseline and AUC:MIC ratio for serum and sputum - All patients

	Spearman's	Change in PFT values from baseline			
Study Day AUC:MIC	rank correlation	FEV_1	FEV_1 % predicted	$FEF_{25-75\%}$	FVC
Day 7 serum	r_s^2	0.072	0.0066	<0.0001	0.021
	p value	0.21	0.71	0.97	0.51
Day 14 serum	r_s^2	0.046	0.0073	0.00018	0.0012
	p value	0.31	0.69	0.95	0.87
Day 7 sputum	r_s^2	0.033	0.040	0.0085	0.19
	p value	0.46	0.41	0.71	0.06
Day 14 sputum	r_s^2	0.025	0.052	0.0053	0.06
	p value	0.51	0.35	0.77	0.31

TABLE 5

Relationship between change in log_{10} CFU and AUC:MIC ratio for serum and sputum - All patients

Study Day AUC:MIC	Spearman's rank correlation	Log_{10} CFU
Day 7 serum	r_s^2	0.021
	p value	0.048
Day 14 serum	r_s^2	0.20
	p value	0.048
Day 7 sputum	r_s^2	0.017
	p value	0.64
Day 14 sputum	r_s^2	0.0031
	p value	0.84

While mean change in log_{10} CFU of *P. aeruginosa* from baseline on both Day 7 and 14 was not statistically significant, the correlation between change in log_{10} CFU from baseline at both of these time points and serum AUC:MIC ratio was statistically significant; increases in serum AUC:MIC ratio were associated with decreases in log_{10} CFU. In contrast, this relationship did not hold with sputum AUC:MIC and confirms the large variability in sputum kinetics of Liposomal Amikacin, that is also shown with TOBI (Geller, Pitlick et al. 2002).

The significant relationships between changes in log_{10} CFU and serum AUC:MIC ratio, and between changes in PFT values and log_{10} CFU, and the lack of significant decrease in log_{10} CFU of *P. aeruginosa* during the two weeks of treatment with liposomal amikacin for inhalation suggests that higher doses may be required to be more reliably effective in a large patient population.

Summary of the Phase 1a/2b Study

Two Phase 1b/2a studies using the Liposomal Amikacin 50 mg/mL have been completed. The two studies were similar in design. A total of 24 CF patients (with $FEV_1 \leq 40\%$ of predicted) received 500 mg Liposomal Amikacin daily for 14 days. The drug was administered using a PARI LC Star nebulizer, over a period of two 20-minute inhalation sessions with a 5 minute rest period between sessions. There were 13 patients enrolled in Study land 11 patients in Study 2. Patient demographics were similar, with the exception of *Pseudomonas* MICs at baseline. In Study 1, the mean MIC (μg/mL) was 8 (range 1.5-16) and in Study 2, the mean MIC was 41 μg/mL (range 8-192). The patients enrolled in Study 2 had prior experience with inhalation antibiotics, and per protocol, were permitted to resume treatment with TOBI®/Colistin after Day 28 of the study. The patients in Study 1 were naïve to inhalation antibiotics, and did not receive additional inhalation antibiotics during the follow-up period. The 500 mg dose of Liposomal Amikacin (50 mg/mL) was well tolerated, and in select patients improved pulmonary function and decreased the density of *P. aeruginosa* in sputum. The details of patient demographics for Studies 1 and 2 (combined) are shown in Table 6.

TABLE 6

Patient demographics in studies 1 and 2.

Variable	Mean	SD	Median	Min	Max
Age	23.7	6.96	22.5	14.0	38.0
Weight (kg)	59.1	13.0	58.6	43.4	99.6
Height (cm)	168	8.10	168	155	194
IBW (kg)	61.4	8.99	60.0	47.9	87.7
CrCL (mL/min)	125	20.9	126	76.8	173

All efficacy analyses in these human clinical Phase 1b/2a studies were exploratory in nature. The efficacy endpoints included:

Change from Baseline in density of *P. aeruginosa* (log_{10} CFU/g) in sputum;

Change from Baseline in pulmonary function tests (FEV_1, FEV_1% predicted, FVC, and $FEF_{(25-75\%)}$

Changes in *P. aeruginosa* sputum density, FEV_1, and FEV_1% predicted at Day 14 were identified as the primary efficacy endpoints.

Quantitative culture of sputum samples and subsequent amikacin susceptibility testing of each morphologically distinct *P. aeruginosa* were performed. The MIC of amikacin for the isolates with the highest MIC cultured from each subject at screening and Day 14 was documented. The density (CFU per gram of sputum) of *P. aeruginosa* in sputum was calculated as the log_{10} value for the sum of all morphotypes.

A summary of the baseline characteristics for the combined population (n=24) are shown in Table 7.

TABLE 7

Baseline measurements for patients in Studies 1 and 2.

Variable	Mean	SD	Median	Min	Max
FEV1 (L)	2.38	1.07	2.18	1.15	6.10
Predicted FEV1 % (L/sec)	65.5	18.9	62.5	40.0	119
FEF25-75 (L/sec)	1.71	1.26	1.49	0.55	5.50
FVC (L)	3.32	0.92	3.27	1.67	5.28
Log10 CFU Count	7.05	1.3	7.3	3.51	8.98
MIC (mcg/mL)	35	56	10	2	192

Study 1: In this study CF patients infected with *P. aeruginosa* isolates sensitive to amikacin (amikacin MIC <64 μg/mL), and those subjects naïve to inhaled antibiotics were enrolled. Administration of Liposomal Amikacin 500 mg once daily for 2 weeks showed a mean change in log sum of counts of *P. aeruginosa* from baseline to Day 14 of 1.09 (n=13; 95% confidence interval, 2.09 to 0.09). The reductions in counts were observed in 9 of the 13 subjects. Treatment with Liposomal Amikacin did not result in selection of resistant strains of *P. aeruginosa*. The mean *P. aeruginosa* amikacin MIC was 8.04 μg/mL at Day 0 and 30.79 μg/mL at Day 14. On Day 14, a single isolate in one subject had a non sensitive MIC (>25 μg/mL); all other Day 14 isolates were sensitive to amikacin. No human was hospitalized or received intravenous anti-*Pseudomonas* antibiotics. Additionally, there was improvement in lung function as measured by an increase in FEV_1 from baseline to Day 14 of +260 mL (n=13; 95% confidence interval, +30 mL to +500 mL). The corresponding change in FEV1% predicted from baseline to Day 14 was

+7.32%. Increases in FEV1 were observed in 9 of the 13 subjects. Also noted were increases in $FEF_{(25-75\%)}$ (mean: 570 mL) and FVC (mean: 180 mL).

Study 2: Study 2 was conducted in a population of CF patients who were infected with *P. aeruginosa*, and were inhalation antibiotic treatment experienced. In these patients, the administration of Liposomal Amikacin 500 mg q.d. for 2 weeks did not show any significant change in *P. aeruginosa* density during the study (p-values >0.297 for change from Day 1). The proportion of patients with mucoid *P. aeruginosa* remained constant throughout the study. No statistically significant changes in FEV_1, FEV_1% predicted, FVC, and $FEF_{(25-75\%)}$ were observed after administration of Liposomal Amikacin 500 mg. Nevertheless, trends suggesting improvement in FEV_1% predicted, FVC, and $FEF_{(25-75\%)}$ were observed at Day 7, Day 14 (end of treatment), and Day 15.

Integrated Efficacy Summary: Studies 1 and 2

Data from the combined population of 24 patients in studies 1 and 2 are summarized below in Tables 8, 9, 10, and 11. The microbiologic end-point of change in log CFU of *P. aeruginosa*, demonstrated a reduction in bacterial density in the combined population, but this did not achieve statistical significance. But, when data were analyzed from the inhalation antibiotic naïve patients (study 1), a statistically significant reduction in CFU was observed at end of treatment. Factors that might explain this effect are the inherent variability in sputum samples, the inter-laboratory variability in methodology, and reporting of quantitative microbiology, and the enrollment of patients with higher MICs (including resistant isolates) in study 2. All of the above are further compounded by the small sample size of each study.

Assessment of clinical benefit by measurement of pulmonary function tests showed a statistically significant improvement in lung function as measured by an increase in FEV_1 from baseline to Day 7 of +240 mL (n=23; p-value 0.0024). The effect at day 14 was a 126 mL increase from baseline in FEV1, which was not statistically significant. A corresponding statistically significant increase in FEV1% predicted from baseline to Day 7 was +7.49% (n=24; p-value 0.0002), and at Day 14 was +4.37% (n=24; p-value 0.0285). The improvement in lung function was also noted with the assessment of small airways as measured $FEF_{(25-75\%)}$ at day 7, an increase in +494 mL (n=23; p-value 0.001), and at Day 14, +423 mL (n=24; p-value 0.0162). These data support a clinically meaningful improvement in lung function in CF patients with chronic *Pseudomonas* infection who have received a 14 day course of treatment with Liposomal Amikacin.

TABLE 8

Change in FEV from baseline at various times in all patients.

Time Point	N	Mean	CV	p-value
Day 7 (pre dose)	23	0.24	1.4	0.0024
Day 14 (pre-dose)	24	0.126	2.86	0.1006
Day 21	23	0.073	4.91	0.3397

TABLE 9

Change in % predicted FEV from baseline at various times in all patients.

Time Point	N	Mean	CV	p-value
Day 7 (pre dose)	23	7.491	1.09	0.0002
Day 14 (pre-dose)	24	4.379	2.10	0.0285
Day 21	23	2.713	3.25	0.1544

TABLE 10

Change in FEF_{25-75} from baseline at various times in all patients.

Time Point	N	Mean	CV	p-value
Day 7 (pre dose)	23	0.494	1.26	0.001
Day 14 (pre-dose)	24	0.423	1.89	0.0162
Day 21	23	0.338	2.15	0.0361

TABLE 11

Change in CFU from baseline at various times in all patients.

Time Point	N	Mean	CV	p-value
Day 7	19	−0.154	−7.37	0.5616
Day 14	20	−0.315	−4.42	0.3242
Day 21	20	0.24	5.4	0.4182

Example 2

Phase 1 Clinical Study

Two Phase 1 single dose clinical studies were completed with 20 and 50 mg/mL formulations of Liposomal Amikacin in healthy volunteers and in CF patients, respectively. Six healthy volunteers received a single dose of 120 mg of Liposomal Amikacin and tolerated it well, and exhibited prolonged retention of the radiolabeled liposomes in the lungs, with a measured half-life of 46 hours.

Liposomal Amikacin was administered to CF subjects with chronic *P. aeruginosa* infections in a human clinical Phase I study (Study 3). Single doses of 90 mg (n=6), 270 mg (n=6), or 500 mg (n=4) were administered to CF subjects to evaluate the safety, tolerability and pharmacokinetics of liposomal amikacin for inhalation. A total of 24 patient dosing sessions of a single dose administration of Liposomal Amikacin or placebo by inhalation via the Pari LC Star nebulizer were evaluated. Two serious adverse events were reported (both occurring in placebo group). Both events recovered without sequelae. A total of 41 adverse events (AEs) were experienced by 17 of the 24-patient sessions dosed (71%) during the trial. Of the AEs reported, 10 of the 16 patients (62.5%) who reported adverse events were in the active group and 7 of the 8 patients (87.5%) were in the placebo group. Headache was the most common AE reported in the active group and no patients were discontinued from the study due to AEs. Liposomal Amikacin was well tolerated and safe up to a single dose of 500 mg administered via inhalation.

Additionally, the PK data confirm minimal systemic drug levels, and high sputum levels of drug, and pharmacodynamic modeling estimates long elimination half life presumably due to slow release from liposomes.

Example 3

Phase 2 Clinical Study

The study design is summarized in FIG. **4**. Patients included in the study were CF patients greater than or equal to six years in age with chronic *P. aeruginosa* infections. Patients were off inhaled antibiotics for 28 prior to beginning the study. Patients were stratified by baseline FEV1(% pred) and randomized 2:1 to ARIKACE® (liposomal amikacin) or placebo (1.5% NaCl). Cohort1 received 280 mg and Cohort2, 560 mg of active drug or placebo for 28 d by inhalation with

PARIeFlow® nebulizer, and were followed for 28 d during which no inhaled antibiotics were administered. Safety, pharmacokinetics, Pa sputum density, Quality of Life (CFQ-R) and exacerbation rate were evaluated weekly during the study period of 56 days.

In summary, daily administration of 280 mg and 560 mg liposomal amikacin for 28 days appeared safe and well-tolerated. Administration of liposomal amikacin at 280 mg and 560 mg for 28 days results in a dose-dependent improvement in lung function, which is sustained at least for 28 days after the completion of the dosing. The patients receiving liposomal amikacin experienced fewer pulmonary exacerbations (7.14%) compared to those receiving a placebo (18.18%). Additionally, the time to exacerbation was prolonged in the amikacin groups (41 days) compared to the placebo (19 days). The groups receiving amikacin experienced no pulmonary exacerbations during the 28 day treatment period. Patients receiving liposomal amikacin demonstrated greater clinical benefit compared to the placebo group as measured by improvement in the quality of life CFQR-respiratory scale.

FIGS. **5** and **6** depict graphs showing the change in oxygen saturation from baseline in pediatric patients (ages 6 to 12) compared to placebo. The results demonstrate an improvement in oxygen saturation beginning during the 28 day treatment period and continuing beyond the treatment period. A similar improvement in oxygen saturation was observed in patients over the age of 12 as well.

FIGS. **7***a* and **7***b* depict the change in lung function as measured by the forced expiratory volume (FEV_1) in the placebo group and the amikacin group, respectively, broken down by age groups. Patients in the placebo group show an overall decrease in FEV_1 by day 56, while patients receiving liposomal amikacin consistently demonstrated an increase in FEV_1 both during and up to 28 days after treatment. The placebo group had the following change in lung function values (measured in mL):

TABLE 12

Change in FEV_1 in placebo group.

age	Day 7	Day 14	Day 21	Day 28	Day 35	Day 56
6-12	−79	−88 (117)	26	0 (61)	−6	−4
13-18	87	2 (80)	25	26 (149)	25	−65
18+	102	−22 (150)	46	36 (135)	−24	−56

The amikacin group had the following change in lung function values (mL):

TABLE 13

Change in FEV1 in liposomal amikacin treated group.

age	Day 7	Day 14	Day 21	Day 28	Day 35	Day 56
6-12	173	232 (117)	138	154 (165)	110	178
13-18	136	133 (157)	143	158 (153)	79	44
18+	103	94 (107)	68	46 (95)	29	55

A comparison of the change in FEV_1 from baseline (measured in mL) for all patients in the 560 mg, 280 mg and placebo groups is depicted in FIG. **8**. Again, the data demonstrates a sustained effect lasting as long as day 56 in patients receiving liposomal amikacin, where the effect is even more pronounced in the 560 mg group compared to the 280 mg group. FIG. **9** represents the change from baseline as a percentage. FEV1 increased significantly in the 560 mg group, with a sustained treatment effect of a 224 mL (a 17.6%) increase compared to the placebo at day 56.

The data from the study also demonstrated a significant reduction in CFU's in patients receiving liposomal amikacin compared to the placebo, and this reduction was sustained at least to day 35. The reduction in CFU was more pronounced for the group receiving 560 mg of amikacin compared to the 280 mg group, as seen in FIG. **10**. FIG. **11** depicts the Log CFU change for mucoid strains. These results demonstrate that *P. aeruginosa* density was reduced, as measured by log CFU, in the groups receiving liposomal amikacin, compared to placebo, and this effect was sustained at least to day 35 of the study. Patients with mucoid strains of *P. aeruginosa* also were susceptible to treatment with liposomal amikacin. A 1.2 log CFU reduction was seen in the 280 mg group, and a 2.0 log reduction in the 560 mg group. The reduction was sustained at day 35 of the 560 mg group with a 1.8 log CFU reduction, while the reduction was sustained with a log 0.4 CFU reduction in the 280 mg group.

The pharmacokinetic data revealed high levels of amikacin in the sputum of patients receiving liposomal amikacin, with the mean Cmax (CV) of 3496 (0.973) mcg/g. The mean area under the curve (AUC) value was 13,120 (1.63) mcg/g*hr for the 280 mg group, while the mean AUC was 22,445 (0.831) mcg/g*hr). The serum pharmacokinetic data, on the other hand, demonstrated low systemic exposure to amikacin, with the Cmax mean (SD) of 2.27 (1.58) mcg/mL.

Patients receiving liposomal amikacin also had a reduced frequency and time to pulmonary exacerbation. Table 14:

TABLE 14

Pulmonary Exacerbations.

	ARIKACE ®	Placebo
Patients	3/42 (7.1%)	3/22 (13.6%)
Time to exacerbation (days)	40.6*	19.3

*No exacerbation during treatment period.

As seen in Table 14, the percentage of exacerbations in patients treated with liposomal amikacin (including both the 280 mg and 560 mg groups) was lower compared to the placebo group. Moreover, the time to exacerbation was much longer in patients receiving liposomal amikacin (40.6 days) compared to 19.3 days in the placebo group.

Anti-Pseudomonal rescue treatments was also reduced in patients receiving inhaled liposomal amikacin, compared to the placebo group, as seen in Table 15.

TABLE 15

Anti-Pseudomonal rescue treatment.

	ARIKACE ®	Placebo
Patients	4/42 (9.5%)	3/22 (13.6%)
Time to exacerbation (days)	43.0*	21.3

*No rescue during treatment period.

As seen in table 15, a lower percentage of patients receiving inhaled liposomal amikacin required anti-Pseudomonal rescue treatment, compared to the placebo group.

Additionally, the time before rescue treatment was needed was reduced in the liposomal amikacin patients (43.0 days) compared to the placebo group (21.3 days).

Example 4

Nebulization of Liposomal Amikacin

The aerosol properties of Liposomal Amikacin produced from the eFlow 40 L are shown in Table 15. When compared to nebulizate generated from the LC Star, the mass median aerodynamic diameter (MMAD) values for the eFlow are ~0.5 μm larger. The actual size dependent mass distributions from both ACI (with eFlow) and NGI (with LC Star) cascade impactors for nebulized Liposomal Amikacin are shown in FIG. **1**. Aerosol from the eFlow/ACI measurements was slightly narrower in size distribution than that from the LC Star/NGI. This difference is reflected in the lower mean geometric standard deviation (GSD) (1.66 versus 1.99) which is a measure of the width of the distribution around the MMAD, see values in Table 16. This narrower distribution offsets any potential effect of a larger MMAD and therefore, the amount of nebulized drug in the respirable range (<5 μm droplet size) is comparable for both eFlow and LC Star.

As shown in FIG. **3**, when dosing was extended in this model to 28 days, there were equivalent reductions in CFUs for animals receiving Liposomal Amikacin dosed daily at ~60 mg/kg or dosed every other day at ~120 mg/kg. Nevertheless, this was only seen as statistically significant for the latter group when compared to animals that received 1.5% saline on the same schedules (p=0.24 and 0.03, respectively). In both cases, there was a significant number of animals in the saline control groups that also experienced 2 log reductions in the CFUs. The longer duration (post 14 days) of saline inhalation treatment seemed to enhance the spontaneous ability of rats to clear their lungs of infection and presumably the agar beads which maintain the chronic infection condition. Rats that received Liposomal Amikacin ~120 mg/kg daily for 14 days, were observed for another 14 days, and then euthanized on day 35. Lungs of these animals had bacteria below the limit of detection, as was the case in the group that received tobramycin 60 mg/kg (given twice per day) daily for 28 days, and then euthanized. Data indicate that in this experiment, Liposomal Amikacin administered at 120 mg/kg once a day for 14 days was as effective as tobramycin 60 mg/kg/day (administered twice a day) for 28 days. This result suggests a higher AUC and possibly a prolonged post-antibiotic effect with Liposomal Amikacin at 120 mg/kg.

TABLE 16

Properties of Liposomal Amikacin Nebulized with the eFlow and LC Star Nebulizers.

		Aerosol Droplet Properties			Percent Associated Amikacin	
Nebulizer	Cascade Impactor Used	MMAD (μm)	GSD (unitless)	Respirable Fraction*	Pre-Nebulization	Post-Nebulization
eFlow	Andersen	3.68 ± 0.26	1.66 ± 0.07	72.9 ± 5.5	96.3 ± 2.1%	66.3 ± 5.8%
LC Star	NGI	3.18 ± 0.18	1.99 ± 0.05	74.5 ± 2.6	96.3 ± 2.1%	62.1 ± 7.4%

The Andersen cascade impactor was used at a flow rate of 28.3 L/min, 18° C., and 50% humidity.
The NGI impactor was used at a flow rate of 15 L/min and 5° C. to achieve >60% humidity.
*Percent mass of the nominal drug dose that is less than 5 μm in diameter.

Example 5

Effect of Liposomal Amikacin on *P. aeruginosa* Lung Infections in Rat

The efficacy of Liposomal Amikacin for Inhalation was studied using a model for chronic pulmonary infection (Cash, Woods et al. 1979) where *P. aeruginosa*, embedded in an agarose bead matrix, was instilled in the trachea of rats. This mucoid *Pseudomonas* animal model was developed to resemble the chronic *Pseudomonas* infections seen in CF patients (Cantin and Woods 1999). Rat lungs were inoculated with 10^4 CFUs of a mucoid *P. aeruginosa* strain (mucoid strain 3064) originally isolated from a CF patient. Three days later, 60 mg/kg Liposomal Amikacin (75 mg/mL) was administered by inhalation once daily for 14 doses (Q1D×14) or every other day for 7 doses (Q2D×7) (6 mg/kg per dose). For comparison, tobramycin was administered by inhalation BID for 14 days (30 mg/kg per dose for a total of 60 mg/kg daily). There was a significant reduction in bacterial density in all three treatment groups as compared to the saline control (see FIG. **2**). There were no significant differences in the reduction of $\log_{10}$CFU/lung between the three treatment groups of rats. It should be noted that Liposomal Amikacin (75 mg/mL) administered every other day for 14 days (Q2D×7), which effectively delivered half the cumulative dose of aminoglycoside, was as effective as the daily dosing regimen in this model.

Example 6

A Multi-Cycle Study of Nebulized Liposomal Amikacin (ARIKACE®) in the Treatment of Cystic Fibrosis Patients with Chronic *P. aeruginosa* Lung Infection

A multi-cycle, open-label study was conducted in order to evaluate the long-term effects of multiple cycles of treatment with ARIKACE® (liposomal amikacin). The lipid component of ARIKACE® comprises the neutral lipids DPPC and cholesterol.

Patients 6 years of age and above who were previously enrolled in a placebo-controlled Phase 2 study of ARIKACE® were consented to participate in this multiple cycle, 18-month study. The design of the study is shown in FIG. **12**. Each cycle comprised 28 days of treatment with 560 mg of ARIKACE®, followed by 56 days off drug. ARIKACE® was administered by inhalation with PARI eFlow® nebulizer. Safety, pharmacokinetics, change in lung function, density of *P. aeruginosa* sputum, quality of life, and exacerbation rate were evaluated at regular intervals.

As shown in Table 17, 49 patients were enrolled in the study, and 45 patients completed six treatment cycles. Patient demographics and other baseline data are shown in Table 18.

The FEV_1(% predicted) at baseline was 59.2%.

TABLE 17

Number of patients completing each cycle

Number of Cycles	Number of Patients Completed Cycle (N = 49)*
Cycle 1	48
Cycle 2	46
Cycle 3	45
Cycle 4	43
Cycle 5	41
Cycle 6	45

*Subjects enrolled in the study over 5-10 months

TABLE 18

Patient demographics and baseline data

		All Patients N = 49
Age (yrs)	Mean (SD)	17.4 (6.2)
Gender	Male	20 (40.8%)
	Female	29 (59.2%)
FEV_1 (L)	Mean (SD)	1.871 (0.772)
FEV_1 (% Predicted)	Mean (SD)	59.2 (19.3)
FVC (L)	Mean (SD)	2.693 (1.109)
FEF 25-75% (L/sec)	Mean (SD)	1.336 (0.766)
BMI (kg/m^2)	Mean (SD)	18.425 (3.114)

ARIKACE® administered once daily for 6 cycles was well tolerated, as adverse events were consistent with those expected in a population of CF patients. Additionally, no unexpected adverse events were observed. Overall adverse events are shown in Table 19 and Table 20 shows a listing of all adverse events by descending frequency.

TABLE 19

Overall adverse events

	All Patients (N = 49)
Number of Adverse Events	351
Patients with Adverse Events	48 (98.0%)
Number of Treatment-Related Adverse Events (Probably or Possibly Related)	33
Patients with Treatment-Related Adverse Events	15 (30.6%)
Deaths	0 (0.0%)
Patients with Serious Adverse Events	15 (30.6%)
Patients Interrupting Study Drug Due to Adverse Events	1 (2.0%)

TABLE 20

Adverse events by descending frequency

Event	All Patients (N = 49)
Cystic fibrosis lung	23 (46.9%)
Cough	14 (28.6%)
Nasopharyngitis	14 (28.6%)
Haemoptysis	11 (22.4%)
Productive cough	10 (20.4%)
Rhinitis	8 (16.3%)
Dysphonia	7 (14.3%)
Influenza	6 (12.2%)
Oropharyngeal pain	5 (10.2%)
Pharyngitis	5 (10.2%)
Pyrexia	5 (10.2%)
Respiratory tract infection viral	5 (10.2%)
Abdominal pain	4 (8.2%)
Sinusitis	4 (8.2%)
Throat irritation	4 (8.2%)
Acute respiratory viral infection	3 (6.1%)
Acute Rhinitis	3 (6.1%)
Abdominal pain upper	3 (6.1%)
Blood alkaline phosphatase increased	3 (6.1%)
Headache	3 (6.1%)
Viral rhinitis	3 (6.1%)

The estimated relative change from baseline in FEV_1 to end of treatment (day 28) during cycles 1-6 was 7.9% (95% CI+4.3, +11.5%, $p<0.0001$). This effect was also sustained at the end of the off-treatment period (56 days) during cycles 1-6, as the estimated relative change in FEV_1 was 5.7% (95% CI+3.0, +8.5%, $p=0.0001$, FIG. **13**). A statistically significant increase from baseline in FEV_1(L) of 11.7% occurred by the end of the treatment portion of the 6^{th} cycle ($p<0.0001$).

Additionally, the results indicated that a significant reduction in *Pseudomonas aeruginosa* density occurred, including for mucoid strains. This effect was sustained over the treatment period of 6 cycles, with each cycle including the 56 day off period (FIG. **14**). The estimated change from baseline in $\log_{10}$ CFU over time was −0.58 log, (95% CI−0.21 to −0.94 log, $p=0.0030$).

There was also no significant shift in minimum inhibitory concentration (MIC_{90}) (FIG. **15**).

The results of the study indicated that 560 mg ARIKACE® given once daily for 28 days followed by 56 days off drug for 6 cycles was well tolerated in cystic fibrosis patients. An increase in FEV_1(%) significantly above baseline was observed during the treatment period of each cycle, and was sustained during the 56 day off-drug period.

Example 7

Biodistribution and Clearance of Amikacin and Lipids of Liposomal Amikacin in Rat Lungs after Repeated Inhalation

A study was conducted to determine the biodistribution, retention, and clearance of amikacin and liposomes in rats following multiple inhalation treatments of 90 mg/kg or 10 mg/kg liposomal amikacin (ARIKACE®). Treatment was evaluated by comparing tissue concentrations of amikacin, fluorescence of TAMRA and $DilC_{18}(5)$-DS and by microscopic evaluation of fluorescence in various sections of the lung.

CDIG female rats were randomized into three groups and received either (1) 90 mg/kg via inhalation for 27 consecutive days, (2) 10 mg/kg of liposomal amikacin via inhalation for 27 consecutive days, or (3) no treatment.

On day 28, rats in each amikacin group were administered a mixture of liposomal amikacin and fluorescently labeled liposomal amikacin (amikacin-TAMRA and $DilC_{18}(5)$-DS liposomes; 90 mg/kg) by inhalation. At 0 and 4 hours post

inhalation and at 1, 3, 7, 14, 21, and 28 days post inhalation, lungs, sera, and urine were collected from 3 rats per group. The concentration of amikacin in tissues was determined by immunopolarization assays. Fluorescence was measured by spectraphotometry. TAMRA and $DilC_{18}(5)$-DS fluorescence was assessed in sera, urine, and lungs.

Lungs were separated into 3 individual right lobes and into 3 segments of each left lobe. The right caudal lobes of the lungs were frozen and later cut into thin sections (7 μm) for microscopic analysis. At each time point, unfixed tissue sections were examined for the deposition of fluorescently labeled amikacin-TAMRA and $DilC_{18}(5)$-DS liposomes using a fluorescence microscope. Images of tissues were photographed and examined for differences in fluorescence intensity.

Exposure of healthy adult rats to 28 daily multiple doses of liposomal amikacin via inhalation resulted in the uniform deposition of amikacin and liposomes throughout the lung in both 90 mg/kg and 10 mg/kg groups. The amount of amikacin deposited per gram of lung tissue was dose dependent. For each dose, the amount of amikacin deposited per gram of lung tissue was the same regardless of which lung lobe was evaluated or what section of a lobe was assayed. Amikacin was cleared from the lung uniformly. Liposomes labeled with $DilC_{18}(5)$-DS were cleared more slowly from the lungs than amikacin, resulting in the lack of measureable levels of $DilC_{18}(5)$-DS in the serum or urine. Sequenced microscopic evaluations of lung tissues dosed with dual fluorescently labeled ARIKACE® revealed that the lipid $DilC_{18}(5)$-DS within the liposomes was primarily sequestered in the macrophages within the alveolar spaces and parenchyma during the entire observation period. Amikacin-TAMRA presented as both diffused and macrophage associated fluorescence during the entire observation period.

The results of the study indicated that exposure to multiple doses of aerosolized liposomal amikacin, regardless of the dose, resulted in a uniform deposition of amikacin and liposomes throughout the entire lung followed by uniform clearance of amikacin from the lung of normal rats.

Example 8

Treatment of Cystic Fibrosis Patients with Liposomal Amikacin

The Phase II program was comprised of randomized, double-blind, placebo-controlled, multiple-dose, multi-center trials in CF subjects. Two similar studies were conducted in parallel in Europe (15 sites) and the US (18 sites). Institutional Review Board and Independent Ethics Committee approvals and informed consents were obtained for all study subjects. The studies were performed in accordance with International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki.

Subjects

Patients could continue with other chronic pulmonary therapies (including hypertonic saline, azithromycin, inhaled corticosteroids, bronchodilators and dornase alpha). Those on chronic inhaled antibiotics were to refrain from use for the duration of the study unless they developed new symptoms or reduction in pulmonary functions that in the opinion of their treating physician required treatment with inhaled antibiotics. Exclusion criteria included infection with *Burkholderia cepacia* or non-tuberculous mycobacteria, active ABPA, significant liver, kidney, or other organ disease that in the opinion of the onsite investigator could place the patient at risk for complications related to study participation.

Inclusion criteria included a CF diagnosis based on sweat Cl–>60 mmol/L or two CF-associated mutations in CFTR with at least one organ system manifestation of CF, age ≥6 years, FEV_1≥40% predicted, chronic *Pseudomonas aeruginosa* infection (defined by four positive cultures over two years, including one within three months prior to screening, and one at screening), clinical stability off of inhaled or IV antibiotics 28 days before dosing, and no known sensitivity to amikacin. Additional information regarding study subjects is found in the Supplemental Methods section.

Study Design

In the European study, subjects were randomized to treatment with once daily Arikace® (280 mg or 560 mg) or placebo delivered by the eFlow® nebulizer for 28 days, followed by 28 days off study drug or placebo. Cohort 1 randomized subjects to 280 mg or placebo in a 2:1 ratio, and Cohort 2 randomized subjects to 560 mg or placebo in a 2:1 ratio (final randomization of 1:1:1 for study arms Arikace® (liposomal amikacin) 280 mg, 560 mg, and combined placebo). In the US study, patients were randomized to treatment with once daily Arikace® (70 mg or 140 mg) or placebo delivered by the eFlow® nebulizer for 28 days (randomization of 1:1:1 for the three study arms). After enrolling 19 subjects, a prespecified DSMB safety review recommended amending the protocol to include dosing with 560 mg Arikace® compared with placebo (single dose cohort, 1:1 randomization). Following a subsequent FDA meeting and review of these recommendations, the study was amended to examine 560 mg Arikace® nebulized once daily compared with placebo, with post-dosing monitoring (off antibiotics) for 56 days (weekly visits for the 28 days of treatment and 28 days post-treatment, then visits at Days 42 and 56 off study drug or placebo). Details regarding the open label extension study are included in the Supplemental Methods section.

Study Design-Open Label Extension

Following a review of the safety and pharmacokinetic data, an open label extension study of repeated Arikace® cycles was conducted at the European sites, utilizing a dosing schedule of 28 days of once daily Arikace® (560 mg) followed by 56 days off treatment (six cycles). All subjects enrolled in the extension study had been enrolled in the 28 day placebo-controlled trial (n=49).

Endpoints

Patients were seen weekly for safety and efficacy assessments. The primary study objective was safety and tolerability [assessed by monitoring vital signs, pre to post-dose lung function (acute tolerability), pulse oximetry, adverse events, hematology, clinical chemistry, urinalysis, physical examinations, audiology, and electrocardiograms (ECGs) and chest X-rays as needed]. Secondary objectives included sputum microbiology, *Pseudomonas aeruginosa* sputum density, and MIC50 and MIC90 isolates for amikacin. Additional secondary endpoints included spirometry [Forced Expiratory Volume in one second (FEV_1), Forced Expiratory Flow25-75% (FEF25-75%), and Forced vital capacity (FVC)], need for rescue antibiotics for pulmonary exacerbations, and the CFQ-R (20-22). Pulmonary exacerbations were defined using the Fuchs criteria (23).

Pharmacokinetic Analysis

Blood samples were collected predose, at 0-1 hour and 3-4 hours post dose on Day 1, and predose and at 0-1 hour post final dose on Day 28 to perform PK analysis. In addition, blood samples were taken on Day 35. Sputum samples were obtained one hour post dosing on days 01, 14 and 28.

Concentrations of amikacin in serum were determined by a sensitive and specific liquid chromatography method with tandem mass spectrometry detection (LC-MS/MS) with a

lower limit of quantification of 0.15 mg/L and an inter-day CV % of <6.95%. Concentrations of amikacin in sputum were determined by a similar tandem mass spectrometry detection method (LC-MS/MS) with a lower limit of quantification of 0.1 mg/L and an inter-day CV % of 8.6%. Concentrations in the sputum were then corrected based on the weight of the sample to obtain sputum concentrations in μg/g.

Statistical Analyses

Safety data were analyzed using descriptive statistics only, and efficacy data were analyzed using an analysis of covariance (ANCOVA) model. The total sample size was considered adequate for the evaluation of the study primary objective (safety) and secondary objectives of the study. A minimum of 60 total subjects treated with Arikace® were required to detect an AE rate of 8% with power of 80%. Analyses were performed on all subjects who received at least one dose of study drug (safety population). All models analyzing lung function and *Pseudomonas aeruginosa* sputum density were by repeated measures ANOVA. Analyses of the CFQ-R scales included calculating both the absolute (Study day value—Day 1 value) and relative changes, and scores for each domain were summarized by visit. The absolute change in domain score was calculated from Day 1 (baseline) to Day 15, from Day 15 to Day 28, and from Day 28 to Day 42, and then analyzed by ANCOVA. Day 1 (baseline) scores were used as the covariate. The MCID score for the CFQ-R Respiratory scale in patients chronically infected with *Pseudomonas aeruginosa* (four points) was summarized for changes from Day 1 to Day 15, and Day 15 to Day 28. A responder analysis, categorizing patients into three groups based on the MCID, was conducted using the relative change scores for each assessment period. Patients who improved by four or more points were categorized as "improved," those with a change of <±four points were categorized as "stable," and those whose scores decreased by four or more points were categorized as "worsened." Treatment groups within each cohort were compared using chi-square tests. Additional supportive analyses included Pearson correlation coefficients between changes in CFQ-R Respiratory Symptoms and changes in FEV_1% predicted from baseline values in the 560 mg vs. placebo groups.

Subjects

A total of 105 CF subjects meeting enrollment criteria were screened, randomized, and dosed in one of four Arikace® dose groups or matching placebo (FIG. **16**). Subjects (across the two studies) were randomized to receive 70 mg (n=7), 140 mg (n=5), 280 mg (n=21), 560 mg (n=36), or placebo (n=36). Baseline demographic characteristics (all subjects) are provided in Table 21. Age, gender, body mass index, FEV_1% predicted, and chronic TIS use were generally similar between the different groups, but with somewhat less severe disease in the 140 mg dose group (higher FEV_1 and BMI, all in the US study, n=5), and younger age in the 280 mg cohort (16 years, all in the European study, n=21). When comparing study subjects enrolled in the European and US studies, mucoid *Pseudomonas aeuruginosa* rates were similar (85 and 89%, respectively), while chronic TIS use was lower in the European subjects vs. the US subjects (defined as >three 28 day cycles in the preceeding 12 months, 19% and 35%, respectively). The European cohort was younger [median age of the European patients was 16.5 yrs (+1-6 yrs, SD) compared with 30.5 yrs (+/−8 yrs, SD) for the US patients], with similar lung function [median FEV_1% predicted of the European patients was 65.7% (+/−20%) compared with 65.3% (+/−19%) for the US patients].

TABLE 21

Demographic summary of all CF patients dosed in the European and US trials (mean and SD).

	Arikace ® 70 mg (n = 7)	Arikace ® 140 mg (n = 5)	Arikace ® 280 mg (n = 21)	Arikace ® 560 mg (n-36)	Placebo (n = 36)
Age (yr)	33.1 (9.7)	35.4 (6.0)	16.0 (5.3)	23.0 (12.6)	20.3 (7.7)
Females, n (%)	6 (85.7%)	1 (20.0%)	16 (76.2%)	15 (41.7%)	20 (55.6%)
FEV_1 (L)	1.87 (0.41)	2.88 (0.40)	2.022 (0.79)	2.19 (0.87)	2.13 (0.70)
FEV_1 (% predicted)	59.29 (12.60)	70.40 (10.09)	66.40 (20.00)	66.39 (17.44)	67.86 (19.36)
$FEF_{25-75\%}$ (L/sec)	0.96 (0.52)	1.91 (0.92)	1.60 (0.90)	1.692 (0.933)	1.53 (0.87)
FVC (L)	3.00 (0.60)	4.25 (0.42)	2.80 (1.10)	3.01 (1.20)	3.08 (1.09)
BMI (kg/m^2)	22.97 (1.57)	26.33 (3.19)	18.06 (2.29)	20.38 (4.06)	19.90 (3.46)

Safety and Adverse Event Profile

The overall frequency of adverse events was similar across the Arikace® dose groups and placebo. Acute tolerability was similar, with 2.8% of Arikace®-treated subjects and 11.1% of placebo subjects demonstrating a drop in FEV_1 of >15% within 30 min of drug nebulization. Respiratory events were the most commonly reported type of adverse event in the trial.

TABLE 22

Adverse events occurring in > or = to 8% in Arikace ®-treated patients compared with placebo (European and US trials)

	Arikace 70 mg (n = 7) %	Arikace 140 mg (n = 5) %	Arikace 280 mg (n = 21) %	Arikace 560 mg (n = 36) %	Placebo (n = 36) %
Tinnitus	0 (0%)	0 (0%)	0 (0%)	1 (3%)	2 (6%)
Nausea	2 (29%)	0 (0%)	0 (0%)	3 (8%)	1 (3%)
Chills	0 (0%)	1 (20%)	0 (0%)	2 (6%)	1 (3%)
Fatigue	2 (29%)	1 (20%)	0 (0%)	2 (6%)	1 (3%)
Vessel puncture site haematoma	1 (14%)	0 (0%)	0 (0%)	0 (0%)	1 (3%)
Pyrexia	0 (0%)	1 (20%)	1 (5%)	3 (8%)	3 (8%)
Sinusitis	0 (0%)	0 (0%)	2 (10%)	2 (6%)	3 (8%)
Creatinine renal clearance	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (3%)
Hyperglycaemia	0 (0%)	1 (20%)	0 (0%)	0 (0%)	1 (3%)
Arthralgia	0 (0%)	0 (0%)	0 (0%)	3 (8%)	1 (3%)
Headache	0 (0%)	0 (0%)	2 (10%)	2 (6%)	3 (8%)
Cough	1 (14%)	0 (0%)	2 (10%)	6 (17%)	4 (11%)
Dyspnoea	0 (0%)	1 (20%)	0 (0%)	1 (3%)	2 (6%)
Dysphonia	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0 (0%)
Haemoptysis	2 (29%)	1 (20%)	2 (10%)	1 (3%)	3 (8%)
Lung disorder	2 (29%)	1 (20%)	1 (5%)	9 (25%)	6 (17%)
Pharyngo-laryngeal pain	1 (14%)	0 (0%)	0 (0%)	3 (8%)	1 (3%)
Productive cough	2 (29%)	1 (20%)	3 (14%)	3 (8%)	5 (14%)
Pulmonary congestion	1 (14%)	0 (0%)	0 (0%)	1 (3%)	1 (3%)
Rales	0 (0%)	1 (20%)	0 (0%)	3 (8%)	0 (0%)
Rhinitis allergic	0 (0%)	0 (0%)	2 (10%)	0 (0%)	0 (0%)
Rhinorrhoea	0 (0%)	0 (0%)	0 (0%)	3 (8%)	3 (8%)
Rhonchi	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0 (0%)
Sinus disorder/ congestion	0 (0%)	1 (20%)	0 (0%)	1 (3%)	4 (11%)
Throat tightness	0 (0%)	0 (0%)	0 (0%)	3 (8%)	0 (0%)
Wheezing	2 (29%)	0 (0%)	0 (0%)	3 (8%)	1 (3%)

Table 22 summarizes the adverse events that occurred in >8% of Arikace®-treated patients and placebos. The most frequent adverse events were respiratory in nature occurring in 45% of all Arikace®-treated subjects compared with 39% of placebo-treated subjects. The adverse event profile was generally similar for the placebo and Arikace® treatment groups, with a tendency for more dysphonia reported in the high dose Arikace® group (8% vs 0%). A total of five subjects in the Arikace® treatment groups discontinued study drug (70 mg and 560 mg dose groups—discontinuations due to respiratory events, dysphonia, laryngitis, and tinnitus) compared with one subject treated with placebo (respiratory event).

TABLE 23

Serum and urine pharmacokinetics of amikacin in Arikace ®-treated subjects during the 28 day placebo-controlled trial (European and US data combined).

Day	Dose	Cmax (mg/L)	Tmax (hr)	Amount in the urine (mg)
1	70	0.22 (0.054)	2.05 (1.83)	4.79 (1.43)
	140	0.38 (0.17)	3.01 (1.92)	15.20 (8.74)
	280	0.95 (0.58)	1.05 (0.295)	17.70 (12.30)
	560	1.29 (0.77)	1.30 (1.29)	33.60 (26.80)
14	70	0.27 (0.060)	0.774 (0.31)	7.76 (3.19)
	140	0.45 (0.16)	2.81 (1.81)	21.70 (12.40)
	280	1.28 (1.02)	2.03 (3.75)	27.30 (16.50)
	560	1.95 (1.38)	1.80 (4.09)	46.20 (40.10)
28	70	0.29 (0.026)	1.57 (1.65)	7.13 (4.31)
	140	0.48 (0.21)	2.14 (1.79)	17.70 (8.33)
	280	1.42 (1.45)	.812 (0.43)	25.20 (19.60)
	560	2.40 (1.62)	2.59 (5.60)	49.50 (46.10)

TABLE 24

Demographic information of CF subjects enrolled in the open-label extension study evaluating repeated cycles of once daily 560 mg Arikace ® (mean, SD - all from European sites). Subjects are segregated by their original treatment assignment during the randomized, double-blind placebo controlled trial that preceded the open label trial.

	Arikace 560 mg (n = 33)	Placebo (n = 16)	Total (n = 49)
Age (yr)	17.7 (6.1)	16.7 (6.7)	17.4 (6.2)
Females, n (%)	21 (63.6%)	8 (50.0%)	29 (59.2%)
FEV1 (L)	1.89 (0.77)	1.84 (0.80)	1.87 (0.772)
FEV_1 (% predicted)	59.5 (19.7)	58.5 (19.00)	59.2 (19.3)
$FEF_{25-75\%}$ (L/sec)	1.33 (0.72)	1.35 (0.88)	1.34 (0.766)
FVC (L)	2.71 (1.12)	2.66 (1.12)	2.69 (1.109)
BMI (kg/m^2)	18.65 (3.25)	17.95 (2.85)	18.43 (3.11)

In addition, eight serious adverse events (SAEs) occurred in the Arikace® treatment groups (all were respiratory in nature). These included five events of pulmonary exacerbation with hospitalization, and four occurred following completion of Arikace® dosing (hospitalizations on Days 23, 37, 37, 42, and 77). A total of two placebo subjects were hospitalized during the study, one for a pulmonary exacerbation (Day 43), and one subject was hospitalized twice (migraine—Day 46, and elevated LFTs, Day 79). There were no other clinically significant changes in laboratory findings during the study. No differences were noted between the Arikace® and placebo subjects over the course of treatment and post-treatment observation in audiology testing.

Spirometry

Pulmonary function data from study subjects are summarized in FIG. **17**. FEV1 in the placebo, 70 mg and 140 mg dose groups demonstrated no consistent trends over the 28 day treatment period. In contrast, the 280 mg and 560 mg dose groups demonstrated rapid and sustained increases in FEV1 at the 14 and 28 day time points that were significantly increased compared with pretreatment values and the placebo controls ($p<0.05$). In the 280 mg dose group, the relative change in FEV1 from baseline was higher at Day 28 compared to placebo (0.101 L±0.128 vs 0.011 L±0.101; $p=0.009$), and returned to pretreatment values by Day 56 (28 days off study drug). In the 560 mg dose group, the relative change in FEV1 from baseline was higher at Day 28 compared to placebo (0.081 L±0.161 vs 0.011 L±0.101; $p=0.033$), that persisted through Day 56 (28 days post-treatment, 0.093 L±0.203 vs −0.032 L±0.119; $p=0.003$). A subgroup of patients treated with 560 mg Arikace® or placebo (15 and 7, respectively) were monitored (blinded to treatment) through Day 84 (56 days post-treatment). In the 560 mg group, the change in FEV1 from baseline at Day 84 was 0.002 L 0.105 compared with −0.066 L 0.143 for placebo (p=NS). Patients receiving Arikace® 560 mg also demonstrated a prolonged time to need for rescue antibiotics for pulmonary exacerbations (mean=45.3±21.7 days) compared with placebo-treated subjects (mean=31.4±18.5 days), but this difference did not meet statistical significance. Together, the findings provide evidence that once daily Arikace® (560 mg) produced sustained increases in lung function for 28 days post-treatment, and provide support for investigation of dosing that included extended off-treatment cycles.

Microbiology

Sputum densities of *Pseudomonas aeruginosa* (Colony Forming Units—CFUs) throughout the trial are included in FIG. **18**, demonstrating rapid and sustained reductions (−1 and −2 logs) at Days 14 and 28 for the 280 mg and 560 mg dose groups compared with baseline and the placebo group ($p<0.05$). *Pseudomonas sputum* density also remained reduced relative to baseline and the placebo group for the 560 mg dose group at Day 35 (seven days off of study drug, $p=0.021$). Arikace® treatment was not associated with the emergence of new CF-associated pathogens, and amikacin MIC50 and MIC90 values did not show consistent trends over the 28 day treatment periods relative to placebo (data not shown).

Patient-Reported Outcomes

Results from the CFQ-R Respiratory domain indicated that at Day 28 of treatment, 66.7% of the 560 mg dose group reported clinically significant improvements (MCID increase >four points), while 22% had decreases meeting the MCID threshold ($p=0.006$). In contrast, placebo-treated patients demonstrated similar percentages of patients with increases and decreases in the CFQ-R Respiratory domain between Days 1 and 28 (33.3% and 38.9%, respectively, p=NS). The differences in Respiratory domain responses between the 560 mg Arikace® and placebo group was statistically significant ($p=0.015$). Changes in the CFQ-R Respiratory domain correlated with changes in FEV1% predicted across the dose groups at day 14 ($r=0.26$), day 28 ($r=0.42$), and day 42 ($r=0.34$; $p<0.05$, $p<0.001$, and $p<0.01$ for the three time points.

Pharmacokinetic/Pharmacodynamic Results

Amikacin pharmacokinetics in sputum, serum, and urine are summarized below and in Table 23. Mean concentrations of amikacin (+/−SD) in sputum samples (1 hour post-dosing)

on Day 1 were 1119 mcg/gm (7222, SD), 1632 mcg/gm (603), 1197 mcg/gm (1866) and 3262 mcg/gm (2810) for the 70 mg, 140 mg, 280 mg, and 560 mg dose groups, respectively. Values at Days 14 and 28 were similar [Day 14 values: 70 mg=1012 mcg/gm (1202), 140 mg=1534 mcg/gm (971), 280 mg=1174 mcg/gm (1185), 560 mg=3183 mcg/gm (3130); Day 28 values: 70 mg=753 mcg/gm (619), 140 mg=1468 mcg/gm (1334), 280 mg=1911 mcg/gm (2450), 560 mg=2645 mcg/gm (2878)], indicating that the airway clearance of amikacin was consistent over the 28 day dosing cycle. Serum amikacin levels remained low throughout the trial. Cmax values were 1.29 mg/L (+/−0.77, SD) on day 1 in patients dosed with 560 mg, with a slight increase to 2.40 mg/L (+/−1.62) by day 28 or dosing. T_{max} values also remained consistent across the trial, varying between 1-3 hours in a fashion that was not clearly dependent on dose. The urine detection of amikacin increased in a dose-dependent fashion, and also increased by 25-50% over the course of the 28 day dosing cycle within the dose groups. Pharmacodynamic analyses demonstrated weak but consistent correlations between lung function [FEV_1 (absolute change, and the change in percent predicted) and $FEF_{25\%-75\%}$] and dose at Days 7, 14, 21 and 28 of treatment (the range of r^2 values over these time points were 0.042−0.136; with p values ranging from $p<0.001$ to $p=0.05$).

Open Label Extension

A subgroup of subjects in the European trial (n=49) were enrolled into an open label extension study, evaluating the safety, tolerability, and efficacy of repeat cycles of Arikace® treatment (560 mg daily for 28 days) followed by 56 days off treatment. Baseline characteristics of this group are included in Table 24, and changes in lung function (FEV1 percent predicted), *Pseudomonas aeruginosa* sputum CFUs, and median MIC90 of *Pseudomonas* isolates are shown in FIGS. **19**, **20** and **21**, respectively. Repeat dosing with Arikace® was well tolerated, with four subjects discontinuing study drug over six cycles, and 15 subjects experienced SAEs (pulmonary exacerbations) requiring treatment with rescue antibiotics.

Forty-eight of the 49 subjects experienced at least one adverse event (AE), with approximately one third experiencing an SAE (none related to study drug). The majority of AEs (59%) reported were mild, and most were classified as either infectious or respiratory. Of the 351 total AEs reported, 33 were categorized as possibly or probably related to study drug. Two subjects discontinued treatment during the open label extension (one randomized to Arikace® and one to placebo during the blinded trial), and there were no deaths. FEV_1 (% predicted—FIG. **4**) demonstrated rapid and sustained increases over baseline values at each of the treatment cycles, with an estimated mean increase of FEV_1 of 7.9 percent predicted points from baseline to Day 28 across all 6 Arikace® cycles (immediately post-treatment, 95% C.I. 4.3% to 11.7%; $p<0.0001$), and 5.7% from baseline to cycle Day 84 (56 days post-Arikace®) across all 6 cycles (95% C.I. 3.0% to 8.5%; $p=0.0001$). *Pseudomonas* CFUs were consistently reduced across each of the treatment cycles [−0.53 Logs at Day 28 of the first treatment cycle ($p<0.025$)], with an estimated change in Log 10 CFUs of −0.60 Logs from baseline over time (C.I. −0.2 to −0.9 Logs, $p=0.003$) across all of the Arikace® treatment cycles, (FIG. **5**). This effect was less pronounced in the final two treatment cycles (p=NS). Median MIC90 values for *Pseudomonas* isolates demonstrated inconsistent increasing trends over the treatment cycles (FIG. **6**, p=NS). The results provide evidence supporting the use of Arikace® in a cycled fashion to suppress *Pseudomonas* spu-

tum density, with prolonged tolerability and sustained improvements in lung function seen over multiple cycles for up to 56 days post-treatment.

Chronic infection with *Pseudomonas aeruginosa* is a common problem in CF that accelerates the loss of lung function, and is an independent predictor of morbidity and mortality in the disease. In the Phase II studies described here, a liposomal amikacin preparation (Arikace®) delivered by a rapid, mesh-based nebulizer was demonstrated to improve lung function, reduce the density of *Pseudomonas aeruginosa*, and to improve patient reported outcomes of respiratory symptoms in CF patients over 28 days of treatment. These improvements were accomplished using a once daily dosing regimen, with improvements in lung function that persisted significantly above that of placebo-treated patients for up to 28 days post-treatment, and up to 56 days post-treatment over multiple cycles relative to baseline values.

The pharmacokinetic and biologic properties of the agent point towards a prolonged treatment effect, with enhanced pulmonary deposition relative to other nebulized antibiotic preparations and enhanced activity at the site of infection in vivo. These observations suggest that cycled treatment strategies that use once daily delivery with this liposomal formulation of amikacin may provide prolonged increases in lung function and reduction in *Pseudomonas aeruginosa* density, potentially reducing treatment burden and enhancing adherence. This is highlighted by the results of the open label extension study, in which the mean FEV_1 showed sustained improvements above pretreatment values over six 28 day treatment cycles followed by 56 day off study drug. These findings contrast with those reported with other nebulized antibiotic preparations approved for the treatment of CF patients infected with *Pseudomonas aeruginosa* (TIS, AI), in which lung function returned towards pretreatment values off-cycle.

Safety and tolerability of Arikace® were supported by the AE and SAE profile observed in the trial, with similar rates of drug discontinuation across the Arikace® dose groups and placebo-treated subjects over 28 days of treatment. There were no differences in audiologic or renal safety outcomes for the Arikace® treatment groups relative to the placebo, which are relevant for the development of nebulized aminoglycosides for chronic use. The amikacin MIC90 of *Pseudomonas* isolates from the Arikace® treated subjects in the randomized and open label extension study did not show an appreciable increase over single or numerous treatment cycles. While longer and larger trials of Arikace® will be necessary to confirm these findings, the results of this trial are similar to those reported with TSI, and provide reassurance for the design and execution of definitive trials examining these clinically relevant endpoints. Furthermore, the relevance of bacterial resistance defined by serum breakpoints is unclear for nebulized antibiotics use in CF, where local airway antibiotic levels typically far exceed defined resistance values and do not predict clinical response.

Encapsulation of aminoglycosides in neutral liposomes for nebulization has several potential benefits relevant to the treatment of CF lung infections. First, aminoglycosides are highly positively charged and have been shown to have electrostatic interactions with CF sputum that limit their bioavailability and killing effects in *Pseudomonas* biofilms. Encapsulation in neutral liposomes can limit these interactions, potentially increasing the delivery of antibiotic deep into CF sputum and biofilms, bringing drug closer to the site of bacterial infection. Second, developing liposomes of an appropriate size may ensure that aqueous phase drug can penetrate the physical barriers characteristic of sputum. Previous stud-

ies have shown that the diameter of Arikace® liposomes (approximately 300 nm), allows their penetration of the natural meshwork of CF sputum, which typically excludes molecules >400 nm in diameter. In contrast, very small diameter agents can pass completely through the CF sputum 'sieve', thus defining an optimal range for small molecule targeting of infected CF sputum. Third, the liposomal components of Arikace® (DPPC and cholesterol) are the same as those found in lung surfactant, and thus are incorporated into the lipid pool used for surfactant recycling. Surfactant lipids are normally cleared rapidly by several pulmonary mechanisms, including type II pneumocytes, Clara cells, and macrophages. The turnover rate for DPPC has been estimated to be approximately 79 mg/hour in the adult human lung, and that of cholesterol is at least 24 mg/day. These clearance rates may also increase with exogenous lipid. It is estimated that the amount of lipid delivered to the lungs following a single 560 mg Arikace® dose (via eFlow with 30% drug deposition) is approximately 73 mg of DPPC (with 29 mg to the lung periphery) and 36 mg of cholesterol (with 14 mg to the lung periphery). Thus, the lipid doses are projected to be normally incorporated into the surfactant pool. Repeated dosing over prolonged periods in animal models has been shown to have little lasting effect on lung histology or pulmonary macrophage function including cytocidal killing. Finally, *Pseudomonas* byproducts (including rhamnolipids) have been demonstrated to disrupt Arikace® liposomes, thus allowing the release of amikacin in the local region of bacterial infection. Together, these characteristics provide a number of novel features that make Arikace® unique among nebulized antibiotics in use or in development to treat CF lung infections, and may provide a new paradigm for pulmonary drug delivery that optimizes drug delivery and deposition while simplifying drug dosing, potentially translating into enhanced adherence and efficacy.

The pharmacokinetics and pharmcodynamics of Arikace® were also unique relative to other nebulized antibiotics used to treat CF lung infections, demonstrating high sputum amikacin levels, low systemic levels, and dose-dependent relationships between Arikace®, FEV_1 and $FEF_{25\%-75\%}$ improvements. The pharmacokinetic data summarized in Table 3 supports prolonged deposition of amikacin in the lung, with stable airway clearance but increasing systemic clearance of amikacin with increasing Arikace® exposure. The consistent reductions in *Pseudomonas* density (FIG. **20**), coupled with prolonged improvements in lung function for up to 56 days off treatment seen over repeated cycles of treatment (FIG. **19**) support a prolonged antimicrobial effect of Arikace®. The MIC90 for *Pseudomonas* isolates in later cycles was increased relative to isolates from earlier cycles, but did not demonstrate a clear trend over six Arikace® treatment cycles or within individual cycles (FIG. **21**). Monitoring of aminoglycoside resistance patterns will need to be included in future studies of Arikace®, including resistance to amikacin and tobramycin.

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INCORPORATION BY REFERENCE

All of the U.S. patents and U.S. published patent applications cited herein are hereby incorporated by reference.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

I claim:

1. A method of treating a *Pseudomonas* pulmonary infection in a patient in need thereof, comprising administering to the lungs of the patient for at least one treatment cycle, an effective dose of a nebulized liposomal amikacin formulation comprising amikacin encapsulated in a liposome wherein the lipid component of the liposome comprises a phosphatidylcholine and a sterol, wherein:

the treatment cycle comprises an administration period of 15 to 35 days followed by an off period of 15 to 75 days: during which an improvement in lung function is maintained for the patient, for at least 15 days after the administration period ends,

and the effective dose comprises 510 mg to 610 mg of amikacin daily during the administration period.

2. The method of claim **1**, wherein the treatment cycle is followed at least twice.

3. The method of claim **1**, wherein the off period is 15 to 35 days.

4. The method of claim **1**, wherein the off period is of 25 to 75 days.

5. The method of claim **1**, wherein the effective dose is about 560 mg of amikacin.

6. The method of claim **1**, wherein the *Pseudomonas* infection is a *P. aeruginosa* infection.

7. The method of claim **1**, wherein the patient has a serum C_{max} of amikacin of less than about 10 mcg/mL during the administration period.

8. The method of claim **1**, wherein the patient has a sputum C_{max} of amikacin of at least 1000 meg per gram of sputum.

9. The method of claim **6**, wherein the sputum C_{max} of amikacin is at least 1000 mcg per gram of sputum during the administration period.

10. The method of claim **6**, wherein the sputum C_{max} of amikacin is at least 1000 meg per gram of sputum for at least 15 days after the administration.

11. The method of claim **6**, wherein the patient has a reduction in $\log_{10}$ CFU of the *P. aeruginosa* infection in the lungs of the patient of at least 0.5 for at least 15 days after the administration period ends.

12. The method of claim **11**, wherein the reduction in the $\log_{10}$ is at least 1.0.

13. The method of claim **1**, wherein the improvement comprises an increase in FEV_1, an increase in blood oxygen saturation, or both.

14. The method of claim **13**, wherein the patient has an FEV_1 that is increased by at least 5% over FEV_1 prior to the treatment cycle.

15. The method of claim **13**, wherein FEV_1 is increased by about 5 to about 50%.

16. The method of claim **13**, wherein FEV_1 is increased by about 25 to about 500 mL over FEV_1 prior to the treatment cycle.

17. The method of claim **13**, wherein blood oxygen saturation is increased by at least 1% over oxygen saturation prior to the treatment cycle.

18. The method of claim **1**, wherein the time to pulmonary exacerbation in the patient is about 20 days or longer.

19. The method of claim **1**, wherein the time to rescue treatment is about 20 days or longer.

20. The method of claim **1**, the lipid component to amikacin weight ratio is about 0.3 to about 1.0 by weight.

21. The method of claim **20**, wherein the lipid component to amikacin weight ratio is about 0.5 to about 0.7.

22. The method of claim **1**, wherein the phosphatidylcholine is egg phosphatidylcholine (EPC), soy phosphatidyl choline (SPC), hydrogenated egg phosphatidyl choline (HEPC), hydrogenated soy phosphatidylcholine (HSPC), dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), palmitoylstearoylphosphatidylcholine (PSPC), or a mixture thereof.

23. The method of claim **22**, wherein the sterol is cholesterol.

24. The method of claim **23**, wherein the phosphatidylcholine is dipalmitoylphosphatidylcholine (DPPC).

25. The method of claim **24**, wherein the liposomal amikacin formulation comprises dipalmitoylphosphatidylcholine (DPPC) and cholesterol in about a 2 to 1 ratio by weight.

26. The method of claim **25**, wherein the liposomal amikacin formulation has a lipid component to amikacin weight ratio of about 0.3 to about 1.0 by weight.

27. The method of claim **26**, wherein the lipid component to amikacin weight ratio is about 0.5 to about 0.7 by weight.

28. The method of claim **6**, wherein the *P. aeruginosa* pulmonary infection is a chronic *P. aeruginosa* pulmonary infection.

29. The method of claim **1**, wherein the human patient is a cystic fibrosis patient.

30. The method of claim **6**, wherein the human patient is a cystic fibrosis patient.

31. The method of claim **1**, wherein the *Pseudomonas* pulmonary infection is a *P. paucimobilis, P. putida, P. fluorescens*, or a *P. acidovorans* pulmonary infection.

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